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EDITORIAL

INFERTILITY IN BULLS

To-day as never before at any time there is need to increase our animal production. The breeding of cattle has always been one of the outstanding features of British agriculture, and the demands for bulls from this country for use throughout the Empire and in other countries has been well maintained over many years. Although breeding in some herds may be satisfactory, there is definite need for improvement in others. It appears to have been accepted in the past that breeding difficulties are to be expected and that, unless they attain a marked degree, they do not call for special attention. Within recent times, however, livestock breeders have shown much more interest in breeding problems and the veterinary profession has been able to be of assistance to them. Whether breeding troubles have increased during recent years is a debatable point: we read from time to time that such is the case. We must not, however, rule out the interest that is now being shown in the subject and the effects of such interest. Be that as it may, we are well aware that infertility, especially of a temporary nature, exists in many parts of the country and is hampering the increased production of cattle to some extent.

Infertility in cows and heifers has receievd much attention during the past few years and some of the causes are now well understood and control measures are being practised with good results. We know, of course, that more and more research work must be carried out in order that we may appreciate the true nature and causes of female infertility which fail to respond to the types of treatment now in use. We have still far to go before the picture will be clear, and for these researches the assistance of personnel trained in and familiar with different aspects of scientific work is necessary. Such personnel exists and more is being trained: we can therefore look forward with confidence to an increase in our knowledge concerning these important subjects and to the devising of measures to control them. The discovery of the presence of œstrogenic substances in some plants is an example of recent developments.

It may not always be fully realised how much infertility occurs in bulls.

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Considerable attention has been given to females in the past: similar considerations do not appear to have been given to the males. With the extensive introduction of artificial insemination as a method of breeding, opportunities have been given to study male fertility to a greater extent. The examinations carried out on bulls for use in artificial insemination work are extensive and it is being shown quite clearly that impaired fertility exists to a degree of some importance. There is need for intensive study of the causes of infertility in bulls. Because of the attention that has been drawn to bull infertility as a cause of poor breeding results in some herds, it is now a fairly common procedure for veterinary surgeons to make examinations of bulls and to give opinions on their breeding potentialities. This is all to the good, but veterinary surgeons will be the first to admit that our knowledge on the subject is very incomplete and that much more information is required in order to assist them in formulating opinions and to prescribe methods for treatment and control. Some research workers have been attracted to the problem and are making good progress in their studies. The subject, however, is of comparatively recent interest and there is, as yet, a scarcity of personnel engaged on it. This will probably be remedied as time goes on when more young men and women develop the necessary interest and become available, by special training and experience, to undertake research work along the necessary lines. The subject is not an easy one to tackle: it has many aspects and its study will require workers trained along various scientific lines. The importance of the work, however, cannot be over-emphasised, for without fertile bulls which will continue to be fertile, breeding operations, be they carried out by natural mating or by artificial insemination, must inevitably suffer.

We draw our readers' attention to an article on the subject of bull infertility, the first part of which is published in this issue of the BRITISH VETERINARY JOURNAL. It represents the results of some careful observations carried out in this country.

GENERAL ARTICLES

CAUSES OF STERILITY IN BULLS IN SOUTHERN ENGLAND

By I. HAQ

Veterinary Investigation Laboratory, Reading.

A. Introduction

ALTHOUGH there has been a considerable advance in establishing new criteria for assessing the fertility of males in recent years, the application of such criteria in determining the specific reproductive disorders in different species of farm animals does not appear to have attracted sufficient attention. As far as has been ascertained, there is no published record of the incidence of reproductive disorders rendering bulls sterile in any part of England. Consequently, an attempt was made to investigate the causes of partial or complete sterility in male bovines in some parts of Southern England. This article deals with the results of the investigations and with the various disorders.

Since the larger the number of semen tests performed the better the assessment of the fertility of a sire, as many tests were made as the facilities permitted. The results show that the tests employed were adequate to diagnose the common genital disorders prevalent amongst bulls. Cases of advanced sterility did not present much difficulty, but those of partial infertility were difficult to diagnose and required elaborate clinical and semen examinations, together with a careful interpretation of the collective findings from history, clinical examination and semen tests. The diagnosis of disease was confirmed after autopsy whenever this was possible. Efforts were made to treat and follow each case to the end, but in some instances it was not possible to collect fuller information.

B. Material and Methods of Diagnosis

Of a total of 41 bulls tested in different parts of Southern England, eight were found to be fertile and the remaining 33 were diagnosed as cases of partial or complete sterility. The bulls in the latter group belonged to Shorthorn (11), Guernsey (10), Friesian (4), Ayrshire (4), Jersey (3) and Hereford (1) breeds. They represented different grades of blood purity and were 1½-9 years old. The methods employed in diagnosing sterility are described below in the order in which they were pursued. Whenever necessary, cows, heifers and other bulls in the herd were also examined to exclude the possibility of attributing the sterility of the herd to the sire under test.

(1) *History*

This was ascertained before taking the bull out of his box; while it was being obtained, the box was inspected and living conditions examined, and the temperament of the bull before handling was examined. Inquiries were made from both the cowman and the owner. Information was gathered mainly with regard to breed, age, age when first put to service, fertility rate (for reasonably healthy cows and heifers since the commencement of the bull's breeding career, including the number of services required per conception), previous diseases and accidents, tests and preventive inoculations, feeding, exercise, parents' health, and the history of the herd in general.

(2) *Examination of preputial washings*

While examining bulls for infertility, it was felt necessary to determine the condition of the prepuce as a routine measure. A clinical examination of the preputial cavity being inadequate for the purpose, a method was devised to determine the condition from a study of the cellular contents of the preputial washings. This comprised an examination of leucocytes and the various types of epithelia arising as a result of surface desquamation or injury to different layers of the preputial mucosa. Such an examination was found very useful, as the clinical examination failed to detect preputial abnormalities in some cases. The method of washing the prepuce and examining its cellular contents has already been reported (Haq and Rollinson, 1948).

Bacteria and moulds were demonstrated by inoculating 5 per cent ox blood agar plates with a loopful of the concentrated washing and examining the same after 24 and 48 hours' incubation. The preparation of subcultures on special media or smears for Gram's staining were resorted to whenever necessary. Diphtheroids other than *Corynebacterium pyogenes,* coliform organisms, hæmolytic and sometimes non-hæmolytic staphylococci, and rarely *B. proteus* were seen, but were considered to be of no significance if the number of colonies of any one type of these organisms was up to 20 per plate. Colonies above that number were taken as an evidence of gross infection. Organisms like *C. pyogenes, Pseudomonas æruginosa* and hæmolytic streptococci were seen on very few occasions. They were considered to be of no significance if any one type of these organisms showed up to two colonies per plate, while the numbers above that figure were taken as an evidence of gross infection.

(3) *Behaviour at service*

The behaviour of bulls is generally described as lively, slow or sluggish. Such a description neither permits a clear comparison of one case with another, nor provides evidence of the psychogenic reactions of a sire at the time of service. In an effort to amplify the usefulness of a description of sexual behaviour, therefore, observations were recorded as under:

"1" or extra lively—on seeing the cow runs and eagerly mounts her.
"2" or very lively—on seeing the cow walks quickly and mounts her instantaneously.
"3" or lively—smells and licks the cow and mounts her within 10 seconds.
"4" or sluggish—smells and licks the cow and mounts her within between 10 and 60 seconds.
"5" or very sluggish—smells and licks the cow and serves her within between 1 and 5 minutes.
"6" or extra sluggish—takes longer than 5 minutes to serve the cow and may have to be walked away from her one or more times.
"7" or impotent—does not serve at all.

Evidence on psychogenic reactions was recorded in regard to (1) libido: display of sexual interest stimulated by the sight or presence of the cow; (2) erection: mode of its onset and protrusion of the penis from the sheath;

(3) mounting and coitus: mode of insertion and copulatory movements; and (4) ejaculation and dismounting; refractory state and desire, if any, to repeat coitus. Information of this kind was of interest in understanding the sexual problems of the bull. There are so many psychological factors responsible for sex maladjustment that it is impossible to understand the ætiological background of a disorder without studying this aspect.

(4) *Semen examination*

Semen was collected from 28 of 33 sterile bulls, the total number of ejaculates examined being 91. The collections were effected by means of an artificial vagina (Hammond *et al.*, 1947) fitted with a liner, cone and tube, steamed and kept plugged with cotton-wool, which was removed just before use.

Immediately after collection, a portion of each ejaculate was removed and put into a sterile tube for bacterial examination; the tube was placed in a thermos-flask containing ice. The remaining portion of the ejaculate was examined for motility and pH, and the tube containing it was wrapped in absorbent cotton-wool (about half an inch thick layer) and ordinary newspaper (four thicknesses) and placed in a thermos-flask with ice all round for transport to the laboratory, which occupied half to three hours. This procedure was adopted to prevent the effect of temperature shock on the spermatozoa (the temperature in the flask fell gradually and remained constant at 4-8° C. for over 24 hours) and to maintain them in a viable condition for the longevity tests. On reaching the laboratory, the samples were taken out of the thermos-flask for 5 to 3 minutes for the preparation of smears and for sperm counting, and were then replaced in the flask for the longevity tests, which extended to a little over 24 hours. Soon after the last longevity test, the tubes containing some of the samples were transferred to ice without wrapping them in cotton-wool or paper and sent for phosphatase estimations.*

The criteria adopted for assessing the fertilising capacity of sperm were those described by Lagerlof (1934), Hotchkiss (1944), Walker and Strauss (1944), Anderson (1945), Lowsley (1945), Marshall and Hammond (1946), Engle (1946 a and b), Williams (1947), Blom and Christensen (1947), and writer's own observations, particularly those relating to tissue cells other than spermatozoa (Haq, 1948 a and b), preputial washings (Haq and Rollinson, 1948), and phosphomonoesterases of semen (Haq and Mullen, 1948). As separate tests were made on sperm density, longevity, bacterial counts, etc., methylene blue reduction tests were not considered necessary.

In order to obtain readily comparable data, 52 ejaculates from 16 fertile bulls at the Artificial Insemination Centre, Shinfield, were also examined simultaneously.

(a) *Volume.*—This was read from the graduated tube attached to the artificial vagina immediately after collection and following jerking the latter in a horizontal position. In order to compensate for the small amount of semen lost in the cone of the artificial vagina used (shorter pattern), the volume recorded in the tube was increased by 0.3 ml. for every ejaculate.

* See Haq and Mullen (1948).

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(b) *Colour and consistency.*—They were noted in daylight by tilting the tube after detachment from the artificial vagina.

(c) *pH* was determined immediately after collection by the use of a capillator (B.D.H. 4-11 indicator).

(d) *Motility.*—This was estimated soon after collection under 2/3 lens (if necessary 1/6), gently warming the slide carrying the semen over a spirit-lamp on cold, windy days. In the laboratory, all estimates were made with the help of an electrically heated stage at body temperature. In order to collect more information than that obtained by the customary method of rating from 0 to 5 (Perry, 1945), it was felt desirable to estimate motility in terms of both the number of motile sperm and the degree of motility, as has been done for the semen of ram (Gunn *et al.*, 1942) and man (Hotchkiss, 1944). The number of motile sperm was expressed as an approximate percentage in tenths of the total number of sperm examined, while the arbitrary numerical values assigned to the latter were as under:

0—immotile.
1—stationary bunting.
2—oscillatory or rotatory movement.
3—progressive movement.
4—vigorous progressive movement with slowly forming waves and eddies.
5—very vigorous progressive movement with abruptly forming waves and eddies.

Thus a motility of 70 x 3 meant that 70 per cent of the total sperm showed a progressive movement and 30 per cent were non-motile, weakly motile, or a mixture of the two. While estimating motility by this method, it is helpful to keep in mind that (a) waves and eddies are usually formed when the percentage of motile sperm is 70 per cent or over; (b) the darker the appearance and the slower the formation of waves and eddies, the greater is the amount of dead sperm and extraneous matter; (c) when only 10-20 per cent of the sperm are motile, the degree of motility most often does not exceed 2; and (d) very thin samples of semen showing sperm prominently and of rather a darker shade generally have a motility between 40 x 3 and 60 x 4.

(e) *Longevity.*—Undiluted semen stored as described previously was examined every six hours up to 24 hours. Observations on the length of sperm survival were discontinued when the original motility ceased to exist or after 24 hours' period, which was taken to be the minimum survival period to ensure fertility under average conditions (Swanson and Herman, 1941). A slight loss of vigour, which accompanied the storage of almost all samples, was not considered to indicate poor longevity.

(f) *Density.*—Counts were made within 1 to 4 hours of collection, using a hæmocytometer (Fuchs-Rosenthall design). Methyl violet 6B was added as a colouring agent to facilitate enumeration (0.1-0.15 ml. of 1 per cent solution in 10 ml. of the diluted semen).

(g) *Abnormal sperm.*—Thin smears like blood-smears were prepared and examined after staining with hæmalum-eosin (Haq and Rollinson, 1948). The

percentage of abnormal sperm was calculated by counting a total of 300 spermatozoa on each smear. Prior to the enumeration of sperm under an oil immersion lens, the smear was examined under lower magnifications to see a general sperm picture and to choose representative parts for final scoring. This procedure is helpful in obtaining more accurate results, as otherwise one may count sperm in a few adjacent fields where abnormalities caused by faulty technique are high. Such parts may exist even on carefully prepared smears. Although the reliability of sperm counts is not affected whether one counts 100 sperm on one smear or 500 sperm each on two smears from the same ejaculate (Salisbury and Mercier, 1945), the practice of counting 300 sperm was adopted to extend observations to more than one field, as a count of 100 sperm in one particular field often failed to show comparatively rare abnormalities which could not altogether be ignored. Occasionally, up to 1,000 sperm were counted to verify the importance of certain abnormalities of rare occurrence.

Abnormalities were recorded in four main groups: those affecting the head, those affecting the middle-piece (with neck), those affecting the tail (from distal end of middle-piece to end of tail), and tailless heads or headless tails (irrespective of morphological changes in the last two cases). A sperm showing more than one abnormality was counted only once, the abnormalities being scored in the order given above. Thus, a sperm showing a coiling of both its middle-piece and tail was scored as one showing an abnormality of the middle-piece and not that of the tail. Total abnormal sperm were obtained by adding figures for the abnormalities of the head, middle-piece and tail to those for tailless heads or headless tails, whichever was higher. (The number of tailless heads tends to equal that of headless tails when many fields are examined.)

(h) *Degenerating sperm clusters.*—These consist of 7-8 spermatozoa (min. 4, max. 42) fused together and showing various degrees of degeneration. The sperm heads are mostly round in shape, measure 2-3 μ (min. 1.7, max. 3.5), and are deeply basophilic. They may, however, appear disintegrated and be irregular in shape, with a markedly eosinophilic staining affinity. The sperm tails are of subnormal or normal length and are either fused together along their long axis or are irregularly intertwined. They can be differentiated from the giant cells (from the testicles) by the presence of completely or partially developed tails and from the clusters of vegetable nuclei by cellular and intra-nuclear structure. Such clusters were recovered from the contents of the genital canal at all levels after slaughter. They were frequently observed in the semen of infertile bulls and were seen in normal semen only on extremely rare occasions.

(i) *Protoplasmic drops.*—They were scored per 100 sperm simultaneously with the enumeration of abnormal sperm. Since they were distinctly visible and conveniently studied with other sperm characteristics on smears stained with hæmalum-eosin (Haq and Rollinson, 1948), observations with dark-ground illumination were not followed as a routine. They were found lying free in the field, in many cases touching the sperm tail and giving the impression of adherence to it. A close adherence to the middle-piece (unshed cytoplasm) was accepted only when a drop was of the same structure and staining affinity as the part of the middle-piece to which it was attached, and when its periphery

could not be discerned. Such a drop took the form of a bleb or collar around the middle-piece and was counted as an abnormality of the latter. A bleb or collar often had a compact structure and a deep acidophil staining affinity, as compared with a free protoplasmic drop which, as a rule, was of a lightly stained, visibly granular, loose texture.

(j) *Tissue cells other than spermatozoa.*—They were scored as described by Haq (1948 a and b).

(k) *Bacteria and moulds.*—The method of culturing and examining semen for micro-organisms was similar to that described for preputial washings.

(5) *Clinical examination*

This comprised external and internal examinations of the genitalia and a general examination of the bull in so far as its reproductive health was concerned. The external examination was carried out to determine the abnormalities of the testicles (particularly the adhesions of the tunic and extent of damage to the testicular tissue), epididymis (particularly the condition of its tail), spermatic cord (particularly the evidence of fat deposition and injuries interfering with the thermo-regulatory function of the scrotum) and penis (particularly the abnormalities of penile muscles, existence of tumours and lesions of trichomonad infection). The internal examination aimed at demonstrating the abnormalities of the terminal part of the vas deferens, seminal vesicles and the intra-pelvic parts of the penis. The normal size, structure and consistency of the various genital organs was regarded as those described by Septimus-Sisson (1941) and Blom and Christensen (1947).

The general examination was especially carried out in bulls with a previous history of accidents and those showing a partial or complete inability to erect the penis or mount the cow. This comprised walking the animal and watching the action of his hind legs and quarters, particular attention being paid to the action of the lumbo-sacral and perineal parts and the hind legs. A manipulation of the affected part was always helpful in verifying the evidence obtained from visual observations.

(6) *Subsequent examination, treatment and autopsy*

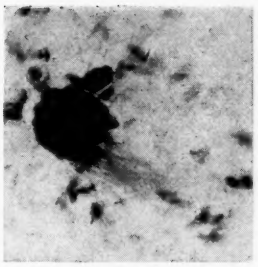
Bulls were re-examined as and when necessary to verify the original findings and to see the results of advice or treatment. In cases of autopsy, the reproductive organs were examined inside the cadaver at the slaughter-house and representative parts were removed from the genitalia for sectioning. The pieces of tissue so removed were fixed in Bouin's fixative and blocks prepared by embedding in paraffin. All sections were cut at a thickness of 5-7 μ and examined after staining in duplicate with hæmalum-eosin and Heidenhein's iron hæmatoxylin.

C. Causes of Sterility and Discussion

Among 33 partially or completely sterile bulls examined (Diagram I), sterility was attributed to testicular degeneration in 19 cases (57.6 per cent), testicular hypoplasia in four cases (12.4 per cent), balanitis in three cases (9.0 per cent), paunchiness in three cases (9.0 per cent), faulty erection in three cases (9.0 per cent), and an unknown disorder in one case (3.0 per cent).

PLATE I

DEGENERATING SPERM CLUSTERS

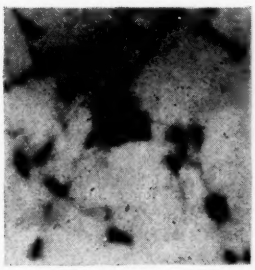


1

2

[x 500.

1.—11 nuclei and over 40 tails partially fused together and all pointing in one direction (AB 33). 2.—A cluster resembling a giant spermatozoon, with 12 nuclei and a thick tail, resulting from the fusion of at least 3 tails (AB 25).



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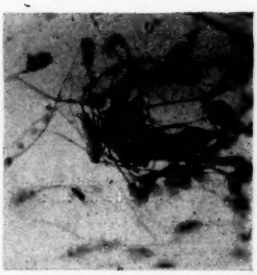

4

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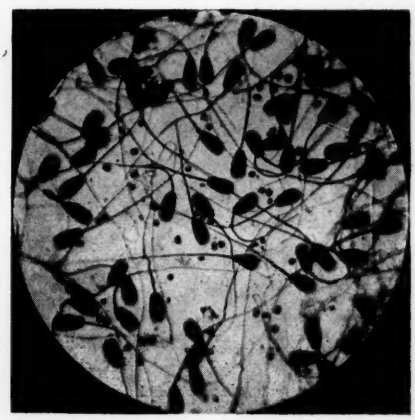
3.—A group of pyknotic nuclei with little tail development (AB 21). 4.—8 nuclei and inter-twined tails (AB 22).

PROTOPLASMIC DROPS



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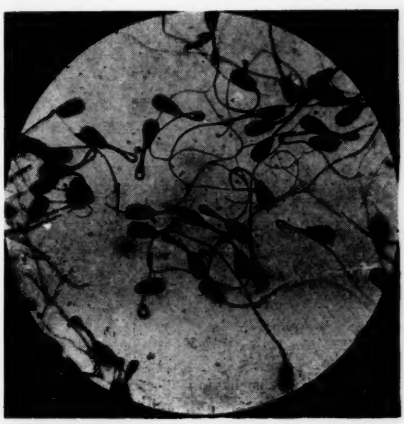

2

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1.—A semen smear showing a large number of free protoplasmic drops (AB 9). 2.—A semen smear showing middle-piece bends around the protoplasmic drops, resulting in poorer motility (AB 27).

(*Article by I. Haq on page* 80.)

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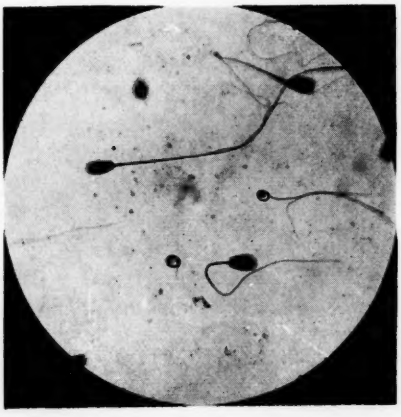

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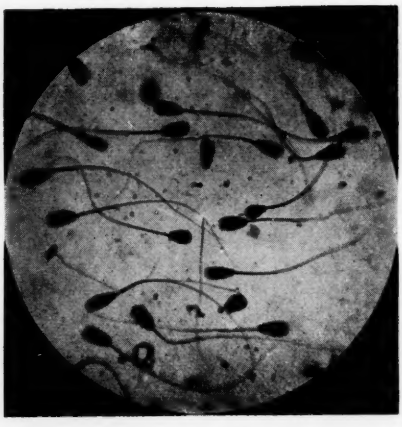
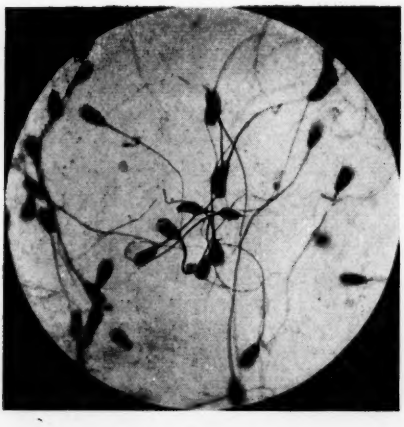


2

1.—A large, deeply stained, basophilic head and two small eosinophilic heads (AB 26). 2.—Small, pear-shaped, basophilic heads on top left, centre and lower third (AB 21). [x 500.



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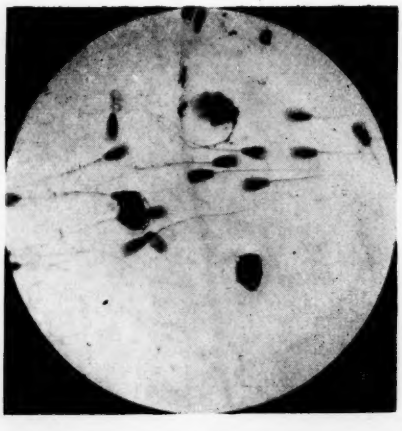
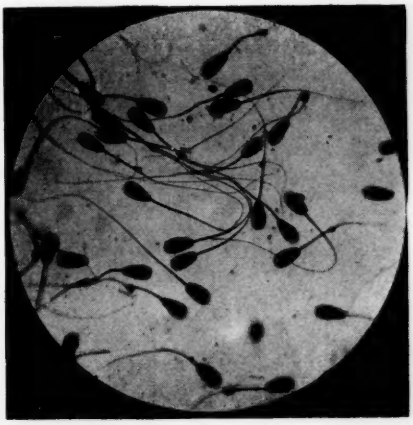


4

3.—Abnormal heads having dissimilar dimensions and showing indifferent staining affinity (AB 32). 4.—Large, degenerated, eosinophilic heads (AB 17). [x 500.

(*Article by I. Haq on page* 80.)

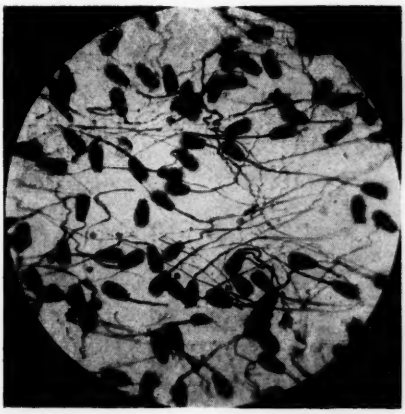
1



2

[x 500.

1.—Bleb or collar near the proximal end of the middle-piece as well as head abnormalities involving their shape and anterior vesicle (AB 25). 2.—Bleb or collar near the distal end of the middle-piece as well as deeply stained and pear-shaped heads (AB 17).



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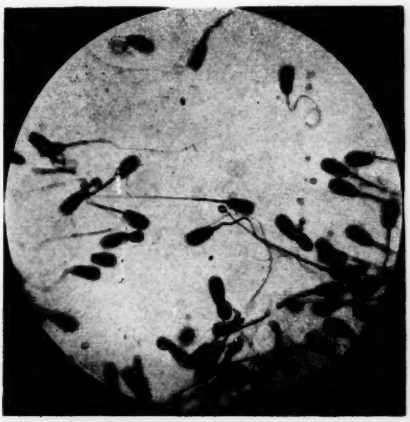

4

[x 500.

3.—Shrivelled middle-pieces (AB 9). 4.—Curled middle-pieces and tails; a sperm lying horizontally in about the centre shows naked axial filament near the distal end of the middle-piece (AB 20).

(*Article by I. Haq on page* 80.)

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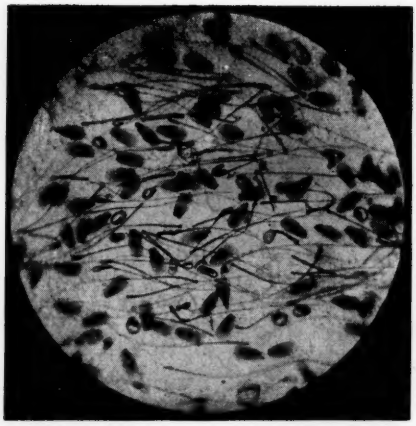

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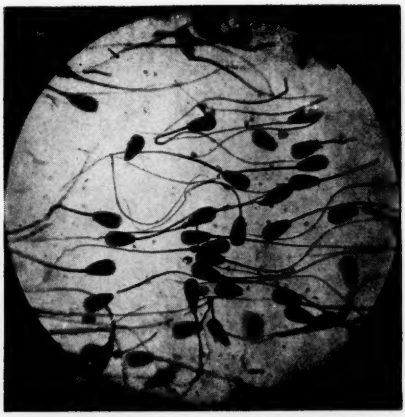
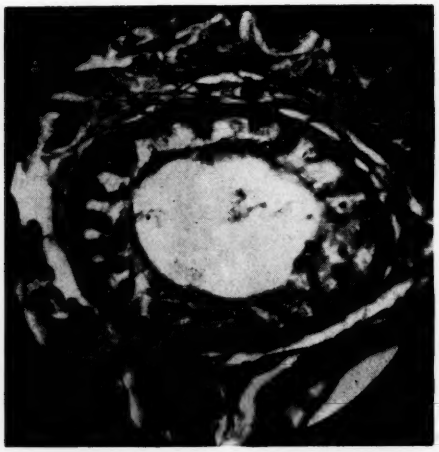


2

[x 500.

1.—An extreme instance of headless tails and tailless heads, with a few disintegrated, eosinophilic heads resembling small rings (AB 33). 2.—Bleb or collar near both the proximal and distal ends of the middle piece (centre), large head (top), deformed heads (top and bottom right), poorly stained head (bottom left), broken neck (top), naked and damaged middle-piece (centre right), curled tails (extreme right), and free heads and tails (AB 31).

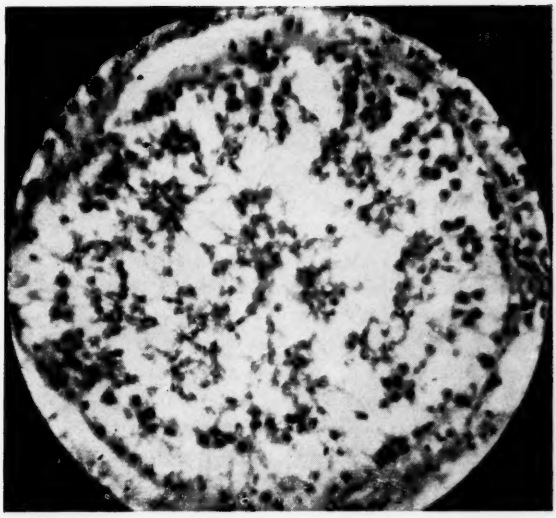


[x 500.

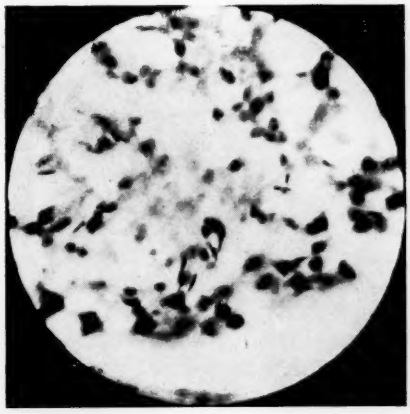
Testicular section showing a hypoplastic tubule lined by only one row of spermatogonia, without any progress towards spermatogenesis (AB 25).

(*Article by I. Haq on page* 80.)

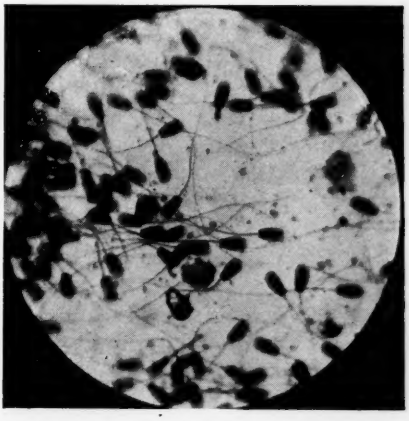
PLATE V



[x 250.
Testicular tubule showing desquamated spermatogenic cells and cytoplasmic granules and masses aggregating in its lumen (AB 15).



1



2

[x 500.
1.—The same section as above under a higher magnification, showing an abnormally large number of cytoplasmic masses of various sizes (AB 15). 2.—A smear of semen or dirty white colour and thick consistency, containing a large amount of cytoplasmic debris (from the testicles) which stood in the way of obtaining a better differentiation in the micro-photograph (AB 15).

(*Article by I. Haq on page* 82.)

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(1) *Testicular degeneration*

This represented the most important disorder and was responsible for interference with the breeding programmes of as many as 19 cases or 57.6 per cent of the total number of infertile sires examined. The figures are slightly higher than those (53.0 per cent) reported by Lagerlof (1938).

(a) *History.*—Case histories brought out three points, viz: poor fertility rate, putting a sire to service at an early age and faulty breeding and management. Fertility rate varied between 0 and 50 per cent—0 in 4, 17-25 per cent in 8, 29-50 per cent in 4, and 58-80 per cent in 3 cases (the rate in the last group comprising AB 21, 31 and 32 was worked out from inseminations performed with selected ejaculates only and was therefore not representative), conceptions occurring to four or more services in many cases. The age at which bulls were first put to service could not be ascertained in all cases. The available records showed that 41 per cent bulls commenced breeding at 9-11 months, 25 per cent at 12 months, 17 per cent at 15-16 months, and 17 per cent at 24 months. Daily ration of concentrates was allowed in an adequate quantity (8-10 lb.) only in 22 per cent cases, the remaining 78 per cent being on improper ration (17 per cent were not fed concentrates at all, 28 per cent received 3-4 lb., 22 per cent 5-6 lb., and 11 per cent as much as 15-18 lb.). Some bulls received concentrates in the breeding season only. The ration of other food materials did not appear to be defective, except that the supply of greens (vitamins) was uncertain and the quantity of hay poor in some cases. Arrangements for exercise were unsatisfactory in three-fifths of the bulls attended, while two-fifths were given hand exercise, tethered in the open or sent out for grazing.

The use of bulls at an early age and faults in feeding and management might not have been responsible for causing testicular degeneration in all cases, but they were considered to have an important bearing on this condition. Although some bulls produce mature sperm at the age of 8-9 months, the seminiferous tubules do not attain adult proportions until about 13 months, the cells of Leydig do not fully develop before 18-21 months and the androgen content does not rise to a high level until about 24 months (Hooker, 1944). The use of bulls at the early age of 9-12 months, therefore, probably results in a strain on the spermatogenic cells and ultimately leads to regressive changes in them. Consequently, breeding bulls before they are about 18 months old should be considered, until more information is available, conducive to testicular dysfunction.

As regards the adverse effects of faulty feeding, it is well known that the degeneration of germ cells sets in when there is a quantitative or qualitative deficiency of proteins and that an adequate supply of proteins is essential to health (Cunningham and Hopkirk, 1935; Albanese and Buschke, 1942; Keller, 1946; Maynard, 1947; Branton *et al.*, 1947). There is some evidence to suggest that the feeding of an adequate quantity and quality of protein in early life is also important, as the monogastric stomach of young ruminants may not be able to synthesise the amino-acids essential for a proper growth of the

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reproductive organs. Once the testes remain in an under-developed condition (not visible macroscopically), it is practically impossible to rectify the defect by feeding large quantities of proteins of diverse composition after the calves have grown into adults. Schaefer (1946) holds the same view. Vitamin A deficiency is also known to cause testicular degeneration in bulls (Erb *et al.*, 1944; Hodgson *et al.*, 1946). In view of the importance of an adequately balanced ration to the reproductive health of bulls, it seems desirable to advise farmers to feed bulls judiciously and to work out adequate standards of feeding sires under different conditions. The average daily ration recommended for a bull during the present investigations comprised concentrates 8-10 lb., silage (preferably roots) 10-12 lb., and hay (preferably seeds or legumes hay) in a sufficient quantity, with greens or outside grazing whenever possible. Stress was always laid on regular exercise for every sire.

(b) *Behaviour at service.*—Keenness to serve did not appear to be related to testicular degeneration. Of the total, 68 per cent bulls showed a behaviour of "1"-"3" and only 32 per cent that of "4"-"7." Signs of sexual weakness were, however, shown in 42 per cent cases by subnormal penile rigidity, erection of glans penis much later than the hinder parts, insertion following prolonged vulvar titilation and protrusion of penis preceded by a few strokes inside the prepuce (this had no significance if it was not a persistent feature).

In view of the important information that each part of the sexual act can furnish, it seems desirable to collect more knowledge on this aspect. Owing in inadequate data, it was not possible to determine the precise origin of each defect, but there was evidence to suggest that they arise from a deficiency of male hormone or injuries. The latter include external injuries to the lumbar (interference with the nerve supply of genitals), perenial (interference with the functions of penile muscles and propulsive mechanism) and preputial (interference with the functions of erectile tissue of the penis and its retractors) regions and injuries to the internal genitalia. Inflammation of the verumontanum, prostatic urethra, seminal vesicles and ejaculatory ducts very often causes sexual weakness in man (Hohman and Scot, 1933).

(c) *Semen examination.*—Semen tests may not detect the precise cause of infertility in each and every case or determine the exact fertility rate of a sire; they can detect abnormalities of the reproductive organs in many more cases than is generally believed. The greater the number and more elaborate the tests, the better the results, the two main essentials being: patience and considered interpretation of the results of collective findings from various tests.

(i) *Volume, colour, consistency and pH.*—Although the average volume of semen (4.3 ml.) in testicular degeneration was lower than that for bulls in the fertile group (4.6 ml.), the difference was not marked. Many bulls ejaculated the same amount of semen and showed a similar increase of volume in the second and third ejaculates as the fertile bulls. The colour was normal in about two-thirds, and greyish white, dull white or dirty white in about one-third cases. Greyish white semen was as a rule accompanied by a low density of sperm. Dull white colour was due either to an admixture with extraneous

matter like vegetable debris and preputial epithelia (e.g. AB 6) or to an abnormally high incidence of spermatids (e.g. AB 33), boat-shaped spermatogenic cells (e.g. AB 8) and cytoplasmic debris from the testicles (e.g. AB 15). Some samples of semen kept at room temperature showed a yellowish-green colour, which intensified progressively with age (from one to four days), owing to their infection with *P. æruginosa* (e.g. AB 21). The consistency was normal in about three-fifths and thin, milky or watery in about two-fifths of the cases. Generally speaking, creamy consistency indicated high (1,000,000-1,200,000 per c.mm.), thin milky low (500,000-600,000) and watery very low (below 300,000) sperm counts. There was also some evidence of increase in the consistency of semen following an abnormal increase in testicular cells and protoplasmic drops or masses. The pH of freshly ejaculated semen in degeneration was slightly higher than that of fertile bulls (6.4 and 6.3 respectively) but did not show outstanding changes.

Samples showing an abnormal colour and consistency suggest a poor quality of semen and should be closely examined to determine the nature of the abnormality present. A dull or dirty white colour can be caused by an increased incidence of spermatogenic cells and protoplasmic drops, there being frequently also an increase in the consistency of semen. Besides the carotinoid pigments (Deakin, 1943), the presence in semen of *P. æruginosa* can also change its colour to yellowish-green, particularly when kept at room temperature.

(ii) *Motility and longevity.*—The average motility in cases of degeneration was 45·5 x 2.7 (min. 5 x 1, max. 70 x 4), compared with 70.8 x 3.7 (min. 10 x 1, max. 90 x 5) in fertile bulls. Only 10 per cent bulls (7 per cent ejaculates) showed motility above 70 x 3 (average requirement of normal semen); the remainder gave semen of poor motility. Judged on the number of motile sperm and the degree of movement separately, poor motility was seen in 90 per cent (93 per cent ejaculates) and 42 per cent bulls (50 per cent ejaculates) respectively. This showed that in most cases of poor motility the defect lay more with the number of motile sperm and not so much with the vigour of sperm movement. Longevity was more than 24 hours in 53 per cent and below normal in 47 per cent ejaculates. Of the latter, 1 per cent lived for 24 hours, 16 per cent for 18, 5 per cent for 12, 16 per cent for 6, and 9 per cent for less than 6 hours. Although semen with poor motility generally showed poor longevity (and contained a large number of abnormal sperm) the converse was not always true, e.g. AB 28 and 33.

In order to be able to obtain comparable data, the use of figures on a standardised system is considered imperative. Owing to a lack of interdependence between motility and longevity in some cases, the importance of performing both tests simultaneously in every case is self-evident.

(iii) *Density.*—The average density was 848,000 sperm (min. 72,000, max. 616,000) per c.mm., compared with 1,076,000 sperm (min. 280,000, max. 1,792,000) in fertile bulls. The number was above 600,000 in 63 per cent, 300,000-600,000 in 26 per cent, and below 300,000 in 11 per cent cases. Another deviation from the normal was a decrease in the number of sperm

with successive ejaculations. Taking individual cases such a decrease was noticed in 42 per cent bulls only, but its intensity being marked reflected itself in the average figures for all the bulls with testicular degeneration. The density decreased from the first to the third ejaculate in the ratio of 100 : 92 : 84 and the volume of semen increased in the order of 73 : 92 : 100 (Diagram II). This decrease appeared to result from the production of a small number of sperm entailing poor reserves and early exhaustion.

The observations shows that (1) sperm density is within normal limits in many cases of testicular degeneration, and hence it is necessary to carry out tests other than density to appraise semen; (2) density below 600,000 sperm per c.mm. should arouse suspicion of genital abnormality; and (3) decrease in density with successive ejaculations very likely indicates the existence of a reproductive disorder. Observations on density below 600,000 sperm and its relation to infertility are more in line with those of Laing (1945) than Lagerlof (1934).

(iv) *Abnormal sperm.*—Table I shows the percentage of abnormal sperm in testicular degeneration and compares the figures with those for testicular hypoplasia and fertile bulls.

TABLE I

Percentage of abnormal sperm in testicular degeneration and hypoplasia

	Abnormalities of						
Disorder	head	middle-piece	tail	tailless heads or headless tails	Total		% age rise over normal
Degeneration ...	7.0	9.3	2.5	12.6	31.4	(min. 15, max. 100*)	108
Hypoplasia	7.5	15.5	1.5	14.6	39.1	(min. 22, max. 58)	159
Fertile bulls ...	2.4	5.9	1.2	5.4	15.1	(min. 6, max. 53†)	—

* This figure was seen only in one ejaculate of AB 33; in other bulls it did not exceed 48.
† This figure was seen in one ejaculate of AB 39 who served for the first time in life; in other bulls it did not exceed 26.

Table I shows that the average number of abnormal sperm in testicular degeneration was about twice that in fertile bulls. Compared with those of hypoplasia, however, the numbers were low: about four-fifths the numbers. These figures were slightly lower than those (degeneration 36 per cent., hypoplasia 42 per cent) reported by Lagerlof (1938). An outstanding increase in abnormalities was noticed in 79 per cent cases of testicular degeneration, while it was within normal limits or only slightly higher in 21 per cent cases. As regards normal semen, although the abnormalities in fertile bulls average 15.1 per cent, in some ejaculates the figure rose to 20 per cent without impairing the fecundity of sperm. Hence the latter figure was taken as an approximate limit of abnormalities for normal semen. This concurs with the findings of other workers (Anderson, 1945).

Abnormalities of different types (Plates I-IV) varied from normal limits to as many as 18 per cent abnormal heads (AB 31), 38 per cent abnormal middle-pieces (AB 16), 26 per cent abnormal tails (AB 20), and 91 per cent tailless heads or headless tails (AB 33). Permissible limits for normal semen

were arbitrarily fixed at abnormal heads 4 per cent, middle-pieces 8 per cent, tails 2 per cent, and tailless heads or headless tails 6 per cent. The division was based on the fact that the semen of bulls in the fertile group did not as a rule show individual abnormalities in excess of these limits, while the semen of bulls in the sterile group gave evidence of deterioration in its quality when the abnormalities were higher than these figures. The incidence of abnormalities of different types and their relationship to other sperm characteristics are shown in Table II.

TABLE II

Different types of sperm abnormalities and their relation to density, motility and longevity

Item	Ser. No.	Bull No.	Total* abnormal sperm (%)	Density per c.mm. (000)	Motility	Longevity (hours)
Head	1	AB 21	32	560	55 x 2.0	15
abnormalities	2	„ 26	28	237	45 x 2.5	6
(32 per cent. cases)	3	„ 28	25	562	20 x 2.0	24
	4	„ 31	32	460	7 x 1.0	6
	5	„ 32	29	572	30 x 2.0	>24
	6	„ 35	27	506	50 x 3.0	>15
	Average		29	483	35 x 2.0	—
Middle-piece	1	AB 9	18	940	30 x 2.0	6
abnormalities	2	„ 15	19	1,025	37 x 3.0	6
(16 per cent. cases)	3	„ 27	32	920	7 x 1.0	<6
	Average		23	962	25 x 2.0	—
Tail abnormalities (5 per cent. cases)	1	AB 14	23	1,412	70 x 4.0	>24
Tailless heads or headless tails (5 per cent. cases)	1	AB 33	100	1,044	32 x 1.7	>24
Combined	1	AB 6	25	741	55 x 3.0	18
abnormalities	2	„ 8	34	576	30 x 2.0	18
(21 per cent. cases)	3	„ 11	25	131	50 x 2.0	<6
	4	„ 20	53	1,512	5 x 1.0	<6
	Average		34	740	35 x 2.0	—
Insignificant	1	AB 12	21†	1,560	65 x 3.0	18
abnormalities	2	„ 16	31†	892	50 x 3.0	>24
(21 per cent. cases)	3	„ 17	17	1,352	55 x 3.0	>24
	4	„ 23	17	680	60 x 3.0	>24
	Average		21	1,112	57 x 3.0	—

* The head abnormalities alone averaged 12 per cent (min. 7, max. 18) in the first group, middle-piece abnormalities alone averaged 18 per cent (min. 8, max. 24) in the second group, tail abnormalities alone averaged 6 per cent (min. 6, max. 6) in the third group, and tailless heads or headless tails alone averaged 89 per cent (min. 87, max. 91) in the fourth group.

† The figure for the second ejaculate did not exceed 16.

Table II brings out a few interesting points: (1) A marked rise in the percentage of abnormal heads entailed poor sperm numbers (below 600,000 per c.mm.) and appeared to indicate defective meiosis, resulting in the production of comparatively few spermatocytes and spermatids. This was borne

out by the number of such cells present in the testicular sections of AB 35. The fact that three out of five cases of prominent head abnormalities showed eosinophilic heads (which indicate more severe degeneration than that denoted by abnormal basophilic heads [Plate II]) gave further evidence of the inability of the spermatids to develop into normal heads (although the tails were normal) as the result of a pre-existing defect inherited from the spermatocytes. It was, therefore, assumed that semen showing poor density and increased head abnormalities, particularly the eosinophilic heads, indicated defective spermatocytogenesis. (2) The abnormalities of the middle-piece, whatever their form, were usually accompanied by normal heads and a normal sperm density (Plate III). This indicated that the process of division of germ cells up to the stage of the spermatids was normal and that the abnormality was produced during the metamorphosis of the spermatids into the spermatozoa. This was borne out by the presence of a large number of spermatids and the co-existing cytoplasmic granules, drops and masses shed during spermiogenesis in the testicular tubules of AB 15 (Plate V). An increase in the abnormalities of the middle-piece, when other parts of the sperm were normal and semen showed a normal density, was, therefore, assumed to indicate defective spermiogenesis. (3) Although the percentage of abnormal sperm with prominent middle-piece abnormalities was lower than the percentage of abnormal sperm with prominent head abnormalities and was accompanied by a higher density, it resulted in a much poorer motility and longevity. This showed that the abnormalities of the middle-piece are as important as those of the head. Whereas an abnormal head fails to fertilise the ovum, an abnormal middle-piece affects sperm movement and does not allow it to reach the ovum. This view is contrary to that held by some workers who seem to have laid too much stress on the abnormalities of the head and too little on those of the other parts of the sperm. (4) An increased incidence of tail abnormalities was seen only in two cases: in AB 23 they were accompanied by a simultaneous increase in abnormal heads, and in AB 20 by a simultaneous increase in abnormal middle-pieces (Plate III). Consequently, definite conclusions were not possible. They could, however, denote: (a) defective spermiogenesis, when found with increased middle-piece abnormalities and a high sperm density; (b) defective spermatocytogenesis, when accompanied by increased head abnormalities and a low sperm density; and (c) mechanical damage or lesions of the seminal canal and accessory organs, when found alone in semen having a normal sperm density. Semen showing abnormalities of more than one type was assumed to indicate defects in spermatocytogenesis, spermiogenesis or both, depending upon the number and type of the abnormalities present. Thus AB 33, whose semen showed extremely few sperm with heads (Plate IV) was a case of disturbed spermatocytogenesis (a few eosinophilic heads with tails were extremely disintegrated) as well as disturbed spermiogenesis (the great majority of the tailless basophilic heads were of normal size and shape and were believed to have separated from the tails immediately after completing their developmental stages; confirmed by observations on testicular sections and other preparations). (6) In cases of insignificant abnormalities of mixed type, AB 17 showed 4-5 per cent degenerating heads

(Plate II). This figure was not high, as even the fertile bulls showed head abnormalities up to 4 per cent. The observations, however, did show that this particular type of abnormality must have affected the fertilising capacity of the sperm, even if the numerical incidence was practically normal.

(v) *Degenerating sperm clusters.*—They were seen only if a large area of the smear was searched. For purposes of convenience they were looked for with tissue cells of semen, i.e. they were searched and scored in an area totalling 43·5 sq. mm. on each flood-smear containing 0.04 ml. of semen diluted to 1 in 5 of normal saline (Haq, 1948b). In fertile bulls, they were observed in 19 per cent cases (18 per cent ejaculates), and in bulls ejaculating them in 45 per cent of the ejaculates searched. In testicular degeneration, they were observed in as many as 79 per cent cases (50 per cent ejaculates), and in bulls ejaculating them in 61 per cent of the ejaculates searched. They were occasionally seen in cases of testicular hypoplasia as well.

The presence of these clusters in semen suggests a disturbance of spermiogenesis arising from the existence of pathological phenomena in the testicles. Such phenomena bear resemblance to those causing a lag-effect in the spermateleosis of the guinea-pig and lepisma observed in testicular degeneration caused by X-rays; the sperm tail elongates in a normal way but the head remains spherical due to a failure of the centrosome to make contact with the nucleus (Gatenby and Wigoder, 1929).

(vi) *Protoplasmic drops.*—The average number of free protoplasmic drops was 5.9 (min. 0, max. 49) per 100 sperm in testicular degeneration, against 10.0 (min. 0, max. 55) in fertile bulls. Since most of the fertile bulls were working at an artificial insemination centre, it appears probable that the increased incidence of these drops in their semen was related to an increased frequency of ejaculations rather than to any specific genital disorder. Even in cases of testicular degeneration there was not enough evidence to establish a relation between the incidence of these drops and infertility. The only harmful effect noticed occasionally in fresh preparations was a mechanical obstruction to sperm tails which curled round the drops and reduced the vigour of the sperm movement (Plate I). In view of the presence of these drops in the semen of fertile and infertile bulls on some occasions and absence on others, it is worthwhile investigating their true origin and relationship to fertility, if any.

(vii) *Bacteria.*—An infection of semen with pathogenic micro-organisms in significant numbers was not observed in any case. The incidence of *P. æruginosa* in testicular degeneration as well as other disorders is described later.

(d) *Clinical examination.*—Of the total, 21 per cent bulls showed no change in the size or consistency of their testicles. Of the remaining 70 per cent, in 26 per cent bulls the testicles were normal in size but had soft and flabby consistency and in 53 per cent they were three-fifths to three-fourths the normal size in addition to a change in their consistency (turgid 26 per cent, soft and flabby 21 per cent, and normal consistency 6 per cent). Soft and flabby texture was accompanied by an increase in sperm head abnormalities (8-18 per cent) in two-thirds cases, e.g. AB 21, 31 and 33. A tucked-up position of the testicles (not due to cold), with a scrotal skin sac formed ventrally, was

seen in two cases: AB 6 showed considerable improvement in semen and testicular position after treatment, while AB 31 did not respond to treatment and showed a deterioration in semen quality and testicular position. An oblique incline in the long axis of the testicles noticed in AB 6 and 14 appeared to be a developmental anomaly of no significance to fertility.

Deviations in the size and consistency of testicles indicate disease. When the size is normal, the consistency proves very helpful in gauging the condition of the testicles. Bulls with testicular degeneration (excluding fibrosis of testicles, which was not encountered in this investigation) may show a normal consistency, but in the majority of the cases (66.6 per cent) it is reduced and the testicles are soft and flabby to feel. The reduction in consistency is often accompanied by a marked increase in the sperm head abnormalities. Lagerlof (1934) found consistency normal or increased, Williams (1947) increased or decreased, and Blom and Christensen (1947) increased in a few instances. As regards the tucked-up position of testicles, it should always call for a more careful search for genital abnormalities, as it may be connected with: (1) deficiency of male hormone, which controls the development and function of the scrotum (Selye, 1943); (2) partial or complete aplasia of organs developing from the Wolffian duct (Blom and Christensen, 1947); and (3) developmental anomalies like short spermatic cord and testicular hypoplasia seen in rams (Gunn *et al.*, 1942) and a few bulls (writer).

In a few cases changes in the size and consistency of the epididymis and seminal vesicles were noticed, but such cases could not be followed to the end to draw definite inferences. In AB 9 an abnormal fat deposition around the spermatic cord and in the proximal part of the scrotum appeared to be the principal cause of degeneration of sperm and their precursors. In AB 21 an abnormally long cord with testicles swinging around like a pendulum was considered to be one of the main causes of interference with sperm livability (putting out of action the thermo-regulatory function of the scrotum), and about six months' rest reduced the size of his cord considerably and this was followed by an improvement in the quality of his semen. The cause of lameness of hind legs noticed in AB 21 and 31 is discussed under faulty erection.

(e) *Autopsy.*—In addition to the various changes characterising degeneration of testicles (Lagerlof, 1934), a histological study of the sections brought out three interesting points: (1) Many nuclei of the spermatogenic cells of AB 11, in which 70-90 per cent of the seminiferous tubules were in a state of degeneration, were pyknotic (atrophied to one-fifth to one-half the normal size) and appeared as bold hyperchromic granules. This was seen in many tubules of both testicles; more in the left than in the right testicle. This may account for 18-21 per cent sperm with subnormal heads (smaller than the other sperm heads by 0.4 μ in length and 0.2 μ in width). (2) In AB 15 the tubular lumina contained an abnormally large number of cytoplasmic masses of various sizes. They were seen in his semen during life and gave it a dirty-white colour and creamy consistency. The basement membrane of the testicular tubules had shrunken and was similar to that reported in the human testis under similar

conditions (Engle, 1946a). There was an extreme breakdown of germ cells, the tubular lumina being hardly discernible (Plate V). (3) The diameter of the seminiferous tubules, taken across the basement membrane, is shown in Table III.

TABLE III

Diameter of seminiferous tubules in testicular degeneration

Serial No.	Bull No.	No. of tubules measured	Right testicle (μ)		Left testicle (μ)	
1	AB 11	18	200	(147-252)	196	(161-260)
2	„ 15	11	205	(168-245)	210	(116-245)
3	„ 33	24	225	(132-310)	209	(120-300)
4	„ 28	28	178	(130-320)	195	(150-242)
	Average	...	202.0	(130-320)	202.5	(116-300)

Figures in brackets give minimum and maximum diameters seen.

Table III shows that the average diameter of seminiferous tubules in the cases studied was 202 μ. In most cases it measured 180-220 μ (min. 116, max. 320), compared with 210-300 μ (min. 175, max. 340) for a normal, three-year-old bull (Haq, 1948). These observations show that the diameter of the seminiferous tubules is reduced in testicular degeneration, but the difference is small and cannot be detected unless close observations are made.

(2) *Testicular hypoplasia*

This disorder accounted for sterility in four cases or 12.4 per cent of the bulls examined. Lagerlof (1934 and 1938) found hypoplasia in 23 per cent bulls. His figure was high owing to the existence of a special type of hypoplasia prevalent among animals of the Swedish Highland breed.

(a) *History.*—All were young bulls 2-3 years old. Fertility rate was 10-40 per cent in one and zero in the other three cases. One bull was made to serve as many as 98 cows in the first year of his breeding career. Faults in feeding and management were the same as already described.

A young, sterile, or very nearly sterile, bull is most often a case of testicular hypoplasia. Heavy use of young bulls should be avoided.

(b) *Behaviour at service.*—It was "1" in three and "2" in one bull. Erection, mounting and ejaculation were instantaneous. The refractory period after ejaculation was extremely short, there being a readiness to serve again before dismounting. Semen was collected in one case by employing a quiet bull in place of a cow. An intensely strong libido displayed by these bulls prevented their owners suspecting sterility for as long as six months to a year.

In sterile herds bred to young, lively bulls, the latter should be suspected for hypoplasia and examined as early as possible to save economic loss.

(c) *Semen examination.*—(i) *Volume, colour, consistency and pH*: The volume was normal with an average of 4.2 ml. (min. 1.0, max. 11.4). Semen was invariably whitish or greyish-white in appearance and had a watery consistency. The pH was higher than normal and averaged 6.8 (min. 6.6, max. 7.0). Two bulls showed a deposit of ammonium urate in their semen (Barron and Haq, 1948).

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(ii) *Motility and longevity.*—Semen contained a few sperm in all the cases examined. They were either dead or showed very poor motility, averaging 6.8 x 0.7 (min. 0, max. 20 x 2). Longevity varied from 6 to 18 hours (35 per

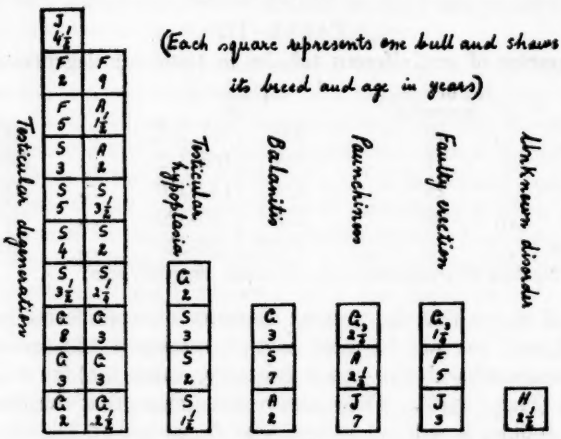

DIAGRAM I.
Comparative incidence of reproductive disorders in bulls in Southern England.
G—Guernsey. S—Shorthorn.
J—Jersey. A—Ayrshire.
F—Friesian. H—Hereford.

cent ejaculates dead, 29 per cent lived for 6 hours, and 18 per cent each for 12 and 18 hours).

Motility and longevity are much poorer in hypoplasia than in degeneration.

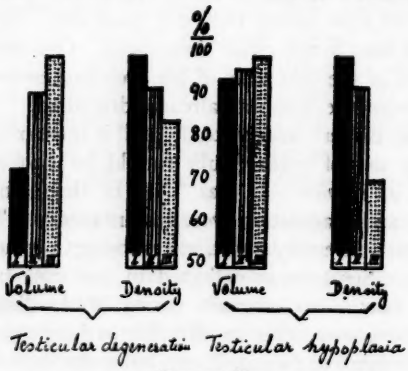

DIAGRAM II.
Increase in volume and decrease in density with successive ejaculations.
I. First ejaculate
II. Second ,,
III. Third ,,

(iii) *Density.*—The average density was 42,700 sperm (min. 0, max. 121,000) per cm., the figures for the first, second and third ejaculates being 48,600, 44,900 and 34,800 respectively. A decrease in sperm counts with successive ejaculations was noticed in all cases. The average fall from the first

to third ejaculate was in the ratio of 100 : 92 : 71, the increase in the volume of semen being in the order of 95 : 98 : 100 (Diagram II). Some ejaculates were milky in colour but contained few or no sperm.

A fall in sperm density with successive ejaculations noticed in hypoplasia further supports the likelihood of poor production and low reserves of sperm in degeneration. The milky colour of an ejaculate does not necessarily indicate the presence of sperm in it.

(iv) *Abnormal sperm.*—As shown in Table III, the percentage of abnormal sperm averaged 39.1 (min. 22, max. 58), and was much higher than the corresponding figure for the cases of degeneration (31.4). One bull showed a marked rise (7-15 per cent) in abnormalities of the head, and the other two in those of the middle-piece (8-36 per cent) and tailless heads or headless tails (14-46 per cent). The eosinophilic heads so often observed in degeneration were not seen in hypoplasia. Abnormalities of the middle-piece mostly comprised collars or blebs around the spiral sheath or tail bends at the caudal end of the middle-piece.

The percentage of abnormalities reported by Lagerlof (1934 and 1938) was 42. The qualitative increase in the various types of abnormalities was similar to his findings, but a corresponding decrease in abnormal heads noticed by him was not observed. On the contrary, in AB 36 the abnormalities of the head were not only much above the normal range but persisted at a high level irrespective of change in other abnormalities. An increased incidence of abnormal sperm in testicular hypoplasia, therefore, does not necessarily imply a normal condition of the sperm heads.

(v) *Protoplasmic drops.*—Free protoplasmic drops were usually not seen; when present they did not exceed 1 per 100 sperm. This very likely resulted from suppressed spermiogenesis and insignificant sperm production, whereby chances of cytoplasmic extrusion and drop formation were considerably reduced.

(vi) *Bacteria.*—The remarks on cases of testicular degeneration are also applicable to those of hypoplasia.

(e) *Clinical examination.*—Deviations from the normal were seen only in the testicles and epididymides. The testicles were, as a rule, one-half to three-fourths the normal size, although in AB 36 one testicle was very small (one-fourth the normal size, descent at 15 months) and the other normal. There was little change in their consistency, except in AB 36, whose one testicle was turgid and the other very soft (fœtal consistency). AB 25 often kept his testicles in a tucked-up position. The epididymides varied from one-fourth to three-fourths the normal size and were occasionally unpalpable.

Small, undeveloped testicles and epididymides of young bulls should strongly suggest the existence of testicular hypoplasia.

(f) *Autopsy.* — As described by Lagerlof (1934), the vast majority of seminiferous tubules were hypoplastic : lined by one or two rows of spermatogonia only (Plate IV). In a few tubules spermatogenesis had progressed to the stage of spermatocytes, spermatids or spermatozoa, but the sperm numbers were extremely small. The tubules invariably contained desquamated germ cells and

giant spermatids. In one case, one-sided vacuolar degeneration of the tail of the epididymis was also noticed (Barron and Haq, 1948).

(3) *Balanitis*

This disorder accounted for sterility in three cases or 9 per cent of the bulls examined. Balanitis was caused by *Corynebacterium pyogenes* in one and by *Trichomonas fœtus* in the other two cases.

(a) *History.* — Fertility rate varied widely. All cows served by AB 7 (*C. pyogenes* infection) failed to conceive and each of them showed vaginal discharge three days after service. AB 19 had a fertility rate of 50 per cent, while AB 29 was standing idle for six months, fertility rate prior to that being 80 per cent (at the time of examination the herd was infected with *T. fœtus*). Feeding and management of these bulls were similar to those previously described.

Herds with a history of vaginal discharge after service or trichomonad infection should call for a detailed examination of the prepuce and penis of the herd bull for bacterial and parasitic invaders.

(b) *Examination of preputial washings.*—Table IV shows the incidence of leucocytes and epithelial cells in the preputial washings of bulls with balanitis, with the corresponding figures for bulls with a healthy prepuce.

TABLE IV

Incidence of leucocytes and epithelial cells in preputial washings

Bull No.	No. of leucocytes per field*	Percentage of preputial cells from: superficial layers	middle layers	deep layers
AB 7	20 (6-40)	97	3	0
„ 19	30 (20-62)	80	18	2
„ 29	5 (1-10)	89	11	rare
„ .. (second visit)	4 (0- 8)	71	25	4
Healthy bulls ...	4 (0-16)	98	2	0

* The figures in brackets refer to the minimum and maximum numbers observed in each case.

Table IV shows that an increase in the incidence of leucocytes denoted an inflammatory condition of the prepuce in all cases. That the inflammation was superficial in the first and deep-seated in the second and third was decided on following cytological evidence.

Gross bacterial infection was found only in AB 7; the other two bulls gave no such evidence. Trichomonads could not be seen in the preputial washings of AB 19 and 29, although they were recovered from the cows served by AB 29. AB 19 was examined only once and AB 29 twice. The clinical symptoms and histories were characteristic of trichomonad infection.

(c) *Behaviour at service.*—This was "1," "7" and "2" in AB 7, 19 and 29, respectively. The refusal of AB 19 to serve the cow was most probably due to: (1) nervousness, as he had always been used to serving in the open and without being led; and (2) the cow employed was not in heat. These observations show that balanitis of trichomonad origin may not necessarily make a bull sluggish at service, as is frequently thought.

(*To be continued*)

VIRUS DISEASES IN THE CAT

By A. R. JENNINGS,
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Part I. Feline Influenza

In a previous paper (Jennings, 1947) the literature dealing with the enzootic diseases of the cat was surveyed. It was concluded there that two main syndromes are encountered, namely, those of feline enteritis and feline influenza or pneumonia. Much has been written, chiefly from the clinical aspect, on feline enteritis, but relatively little on the respiratory disease. Hindle and Findlay (1932) investigated "feline distemper," but did not distinguish the condition from feline enteritis. Dalling (1935), however, recognised the two types and showed that they were immunologically distinct. Baker (1944) described a form of feline pneumonia with lesions in the eyes, nose and lungs. The condition called here feline influenza resembles, in many respects, that described by Baker.

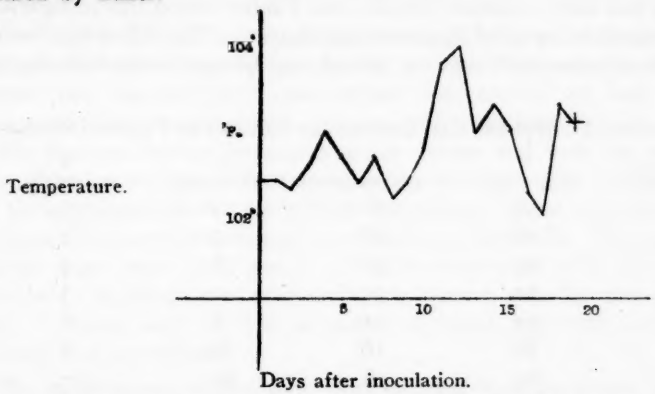

Fig. 1. Temperature chart in a case of Feline Influenza.

The material used in this study was obtained from various sources. Much of the material used for the histo-pathological work was obtained from cases received *post-mortem* from local practitioners and a few clinical cases were obtained from the same sources. An outbreak of the disease in laboratory kittens furnished further cases and some rather limited transmission experiments were carried out.

Clinical Course of the Condition

The disease, as is well recognised, is one affecting young cats; older cats appear to be resistant. The condition is one of fairly long duration; the average time in the cases seen varied between two and three weeks. There was an initial rise of temperature which persisted for two or three days and then dropped almost to normal. Throughout the disease there were minor temperature fluctuations. A typical temperature chart may be seen in Fig. 1 above.

A bilateral oculo-nasal discharge due to conjunctivitis and rhinitis was a constant sign. Affected animals frequently coughed and sneezed (especially after handling) and many animals remained "snufflers" for long periods after an otherwise apparent recovery. The appetite was variable, a few cats showed vomiting and some had an intermittent diarrhœa, but the majority were constipated. There was marked loss of condition and affected cats were listless. The profound depression noted in feline enteritis was, however, absent.

The blood picture in affected animals was studied, particularly in the laboratory outbreak. Daily examinations were made and particular attention paid to the leucocytes. No significant changes were noted in the hæmoglobin and red cell levels. In a few cases there was a slight leucocytosis, but in all cases there was a relative neutrophilia. This was often well-marked; over 70 per cent of the leucocytes were neutrophils, these cases usually showing a "shift to the left." There was a relative eosinopænia and lymphopænia, with a slight monocytosis. A fall in the neutrophil count, in conjunction with the reappearance of eosinophils, was regarded as a favourable sign. Although the monocytosis was fairly constant (Hindle and Findlay noted this in their cases), it was not considered to be of diagnostic significance. The differential leucocyte counts in an experimentally infected animal may be seen in the following table.

TABLE SHOWING DIFFERENTIAL LEUCOCYTE COUNTS IN FELINE INFLUENZA

Days after infection	% neutrophils	% lymphocytes	% monocytes	% eosinophils
2	54	38	3	5
3	56	38	3	3
4	54	36	6	4
5	60	33	4	3
6	73	12	8	7
7	68	16	14	2
8	64	16	20	—
9	60	24	14	3
10	79	13	8	—
11	73	16	10	1
12	76	14	8	2
13	animal destroyed			

These blood findings are quite different from those noted in feline enteritis. In this latter condition there appears, in the cases examined here in Liverpool, a definite reduction in the total white cell count. This fall in the numbers of cells is most marked in the neutrophils and may be most spectacular.

Incubation Period

The period of incubation in feline influenza appeared to vary a good deal. In the laboratory outbreak four apparently healthy kittens aged eight weeks were obtained from a farm. These animals were isolated and the usual pre-

cautions against infection were adopted. So far as is known there was no contact with other cats or with possible infective material. Eleven days after arrival one kitten had a temperature of 104° F. and a slight nasal discharge. (Daily temperature readings were taken.) All four kittens developed the disease between the 11th and 14th day after arrival at the laboratory. Enquiries at the farm revealed that the place had a history of "feline distemper." Healthy kittens artificially infected (by inoculation and contact) showed variations in the incubation time. One cat had pyrexia and a differential leucocyte count of neutrophils 72 per cent, lymphocytes 13 per cent, monocytes 13 per cent, and eosinophils 2 per cent, four days after the inoculation of bacteria-free filtrate. The following day the temperature dropped and the animal was then clinically normal, except for the changed blood picture, until the tenth day after inoculation, when typical symptoms appeared. The shortest incubation period noted was seven days and the longest 14 days. Baker (1944) stated that the incubation period in the condition he described was from 6 to 10 days.

Causal Agent

Previous work (Dalling 1935, Baker 1944) indicated that the condition, or one closely resembling it, was due to a filterable virus. In all the cases presented here bacteriological examinations were carried out both during life and at *post-mortem* examinations. Swabs were taken from the eyes, the nostrils and the pharynx during the course of the disease and from the viscera after death. The bacteriological findings were not constant. The organisms isolated from the oculo-nasal discharges included streptococci (these were mainly Lancefield Group G), staphylococci, and, in one case, a pasteurella. The most constant organism found after death was *B. coli,* with occasionally a few pyogenic cocci. In one field case an untyped salmonella was isolated from the mesenteric lymph nodes. Cultures were made on a variety of media and were incubated both ærobically and anærobically.

For experimental transmission work affected lung tissue and the adjacent lymph nodes from typical cases were suspended in glucose broth and were then filtered through a collodion membrane with an A.P.D. 0.75μ. This bacteria-free filtrate was then checked for sterility and was given intranasally into young kittens, mice, rabbits and guinea-pigs. Control animals were given sterile glucose broth in the same manner. The disease was reproduced in injected kittens (2). Another kitten which had been placed in a separate cage adjacent to a cage containing the two infected kittens developed the infection. The disease developed in this kitten nine days after being placed in contact with the infected kittens. Both these kittens were having bouts of violent sneezing at this time.

Rabbits and guinea-pigs inoculated with the bacteria-free filtrate showed no signs of disease (Baker stated that in his cases, rabbits, guinea-pigs, hamsters and embryonated eggs could be infected from infected cat lung tissue). The mice, however, did develop a respiratory condition which on *post-mortem* examination appeared to be of the influenzal bronchopneumonia type. This

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mouse pneumonia was not always fatal, and, generally, mice died or were killed after they had shown sneezing and nasal catarrh for several days. This mouse pneumonia was passaged, using bacteria-free filtrates as inocula, four times into mice, but at the time no healthy young cats were available so that it was not possible to bring back the virus into cats. Because of this and also because pneumonia appeared in the control mice during the later stages of this work, these mice experiments were discontinued. Unfortunately there are several forms of natural murine pneumonia and this leads to confusion when working with respiratory diseases in this species. The very limited work which was done on mice, however, did suggest that these animals were susceptible to the virus. The lung lesions in the mice resembled those seen in kittens, and, in addition, an enlarged spleen was a constant feature. It is possible that mice may play a part in the epizootology of feline influenza, and it is suggested that further work should be done with this in mind.

Mortality Rate

No treatment of any kind beyond the usual routine of good hygiene and good feeding was adopted. Some cats were killed at various stages of the disease, but the general impression gained during naturally-occurring outbreaks was that the mortality rate was low. This is in marked contrast to feline enteritis in which the mortality rate is high.

Pathology

Cats were examined as soon as possible after death. The lesions were confined to the eyes and the respiratory tract.

Gross appearance.—The animals were in poor condition with little subcutaneous fat. There was usually a bilateral suppurative conjunctivitis; in some cases this suppurative inflammation also involved other structures in the eyeball (*vide infra*). The naso-pharynx was congested and the surface covered with a thick tenacious exudate. In some cases the ethmoid bones were hyperæmic and moist. The posterior portion of the tongue in two cases was reddened with very superficial erosions (Smythe, 1934, described an infectious glossitis in cats). In a few cases there was reddening of the trachea, but generally, cases all had lung lesions of varying degrees of severity.

The apical and cardiac lobes of the lungs were most frequently affected and the lesions, which were patchy in distribution, ranged from an acute congestion to a broncho-pneumonia with consolidation. In one case there was a fairly extensive pleuritis. The smaller bronchi were full of a grey catarrhal fluid. A feature noted, especially on microscopical examination, was the presence of hæmorrhages in the lung substance. The pneumonic areas varied in colour from a dark red, almost black colour, to a dirty greyish yellow. A few areas of emphysema were often present especially at the apices and borders of the lungs. The bronchial lymph nodes were invariably enlarged, excessively moist and congested. The heart and pericardium were normal to the naked eye.

In one case only was there involvement of the gastro-intestinal tract, and this animal had a catarrhal enteritis.

Microscopic appearance.—Portions of the affected organs were taken and paraffin sections prepared. A variety of fixatives and staining techniques were employed. Particular attention was paid to the search for possible inclusion of elementary bodies since this condition is of virus origin, and Baker had reported elementary bodies as a constant feature in the feline pneumonia which he described.

Lungs.—The disease appeared to start as an acute congestion of the lungs, especially in the ventral parts of the apical lobe. This hyperæmia was often accompanied by, or followed by, actual hæmorrhage. This hæmorrhage was sometimes extensive, and it was usually accompanied by alveolar œdema. The type of lesion is illustrated in Fig. 2.

The next stage noted in these cases was a rapidly spreading capillary bronchitis with catarrhal exudation. Some bronchioles were apparently completely plugged by exudate. This inflammatory process then spread as a bronchopneumonia. The alveoli became filled with a cellular exudate containing little fibrin. The cells were, in the main, neutrophils and monocytes. A few areas of collapse were apparent and there were marked vascular changes. In some sections a few peribronchial vessels showed thrombosis. Another feature was the peribronchial accumulation of cells, mainly lymphocytes and monocytes. Resolution was accompanied by a clearing of the alveoli and the bronchial tubes together with an increase in the interstitial fibrous tissue. In some of these cases the alveolar walls were much thickened.

Lymphatic nodes.—The bronchial lymph nodes in section revealed lymphadenitis with some hyperplasia of the lymph follicles. The nodes were congested and there were cellular infiltrations. The cells were mainly monocytes with a few neutrophils present. Some of the lymph sinuses were filled with these cells.

Eyes.—There was, in most cases, a very definite suppurative conjunctivitis. This suppurative inflammation tended to spread, and Fig. 4 shows a section of the cornea of an infected case. The cornea is infiltrated with neutrophils and a fibrinous exudate. There is separation of Bowman's membrane and of the substantia propria. This particular animal was destroyed at the height of the disease and it appears that, if the disease had been allowed to progress, it would shortly have become a case of panophthalmitis.

Other tissues.—Sections were prepared from other tissues, including liver, spleen, kidneys and the intestines. No constant features were noted beyond some congestion and cloudy swelling of the liver.

Inclusion and Elementary Bodies

Inclusion and elementary bodies have been described in very many virus diseases. In the feline diseases, Hammon and Enders (1939) and others have described intranuclear acidophilic bodies, and Baker (1944) described a feline pneumonia characterised by elementary bodies in the monocytes. With these findings in mind particular attention was paid to the possible presence of such structures in these cases of cat influenza. Sections and smears from various

tissues were stained by such stains as Giemsa, Macchiavello's, Goodpasture's and William's. No constantly occurring structures could be found which could be regarded, beyond doubt, as either inclusion bodies or elementary bodies In some cases smears from the bronchial exudate stained by a slow Giemsa stain revealed clusters of rather basophilic structures in the monocytes, but these were not found constantly and rarely were they present in sections of lungs. In some cases these structures were found outside the cells in the smears. It may be that insufficient number of cases have been examined, but to date structures such as those described by Baker have not been found.

Summary

A very brief account of the condition called feline influenza is given. It appears to be of virus origin and differs in many ways from feline enteritis. No characteristic changes in the blood were noted. The pathology of the lung lesions is described.

Part II. Feline Enteritis

In the preceding paper the condition termed feline influenza was discussed. It is the purpose of this communication to describe briefly a few cases of another enzootic disease of the cat, namely, feline enteritis. The two conditions give rise to much confusion since they bear some clinical resemblance to each other and also the two conditions may be present simultaneously during outbreaks of disease in catteries and similar places.

The cases which form the basis of this work were obtained largely through the courtesy of the local veterinary practitioners. Much of this material was obtained *post-mortem* so that opportunities were not available for making detailed clinical and blood examinations. Wherever possible, however, these examinations were carried out.

Clinical Course

Generally, the condition was of sudden onset and ran a rapid course. Contrary to the general description given, vomiting and diarrhœa were seldom seen. The most marked feature of the cases noted was a profound depression which was gradually succeeded by coma from which the animal failed to recover. There was complete loss of appetite and the affected cat often adopted a characteristic attitude, crouching over its untouched food or drink. The coat was usually harsh and staring and the eyes sunken. The abdomen was tense and painful on palpation. There was no oculo-nasal discharge and no coughing or sneezing. There was an initial rise of temperature followed by subnormal temperature and death. It is the combination of these symptoms, especially the sudden onset and the profound depression, which is responsible for the inevitable history given by the owner that " the cat has been poisoned."

Blood Changes

An infectious disease of cats, the chief feature of which is a marked change in the leucocyte picture, has been described independently by American and Continental workers. The condition has been termed variously malignant pan-

PLATE I.

FIG. 2
Section of lung showing hæmorrhage and œdema. H. & E. x 200.

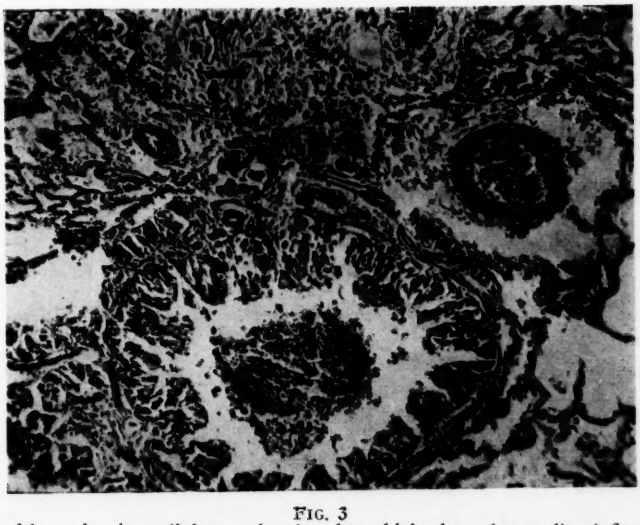

FIG. 3
Section of lung showing cellular exudate in a bronchial tube and spreading inflammatory change in the adjacent alveoli. H.E. x 200.

(*Article by A. R. Jennings on page* 89.)

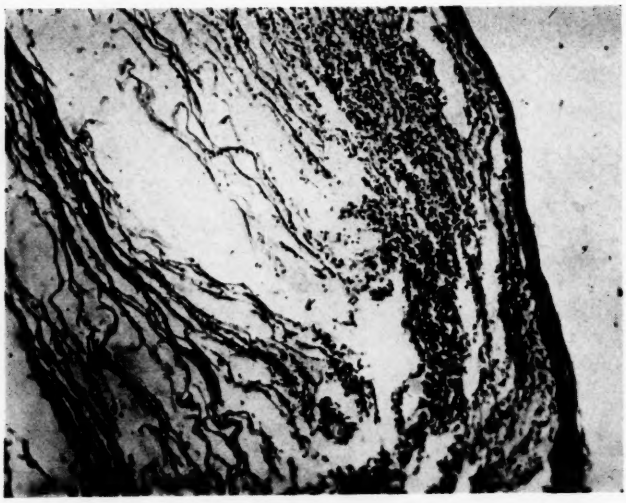

FIG. 4
Inflammation involving the cornea, with neutrophilic invasion and separation due to exudate. H. & E. x 200.

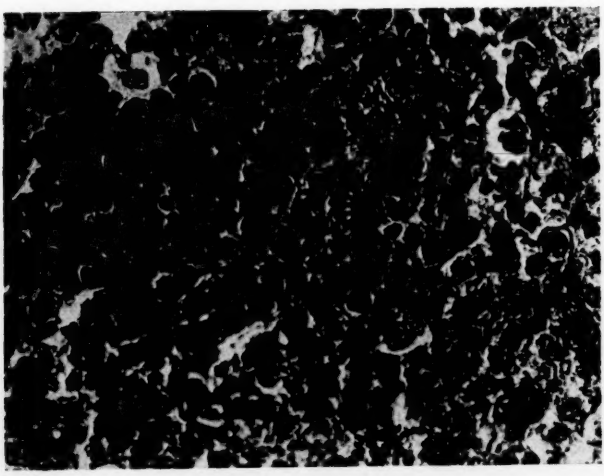

FIG. 6.
Spleen showing eosinophilic bodies inside the nuclei of large mononuclear cells.
Good pasture x 400.

(*Article by A. R. Jennings on page* 89.)

leukopænia of cats, feline agranulocytosis and fulminating feline panleukopænia. It bears a close resemblance in many ways to feline enteritis, but, so far as is known, the two diseases have not been shown to be identical. Lawrence, working with Syverton (1943), drew attention to the close similarity of these two feline diseases but stated that there had been no record of significant blood changes or of the typical inclusion bodies. As will be seen, definite and significant blood changes were noted in these cases of feline enteritis.

The American workers, while agreeing in the main, disagree somewhat in their interpretation of the blood changes. Hammon and Enders (1939) ascribed the condition to a failure of leucopoiesis with a resulting panleucopænia,

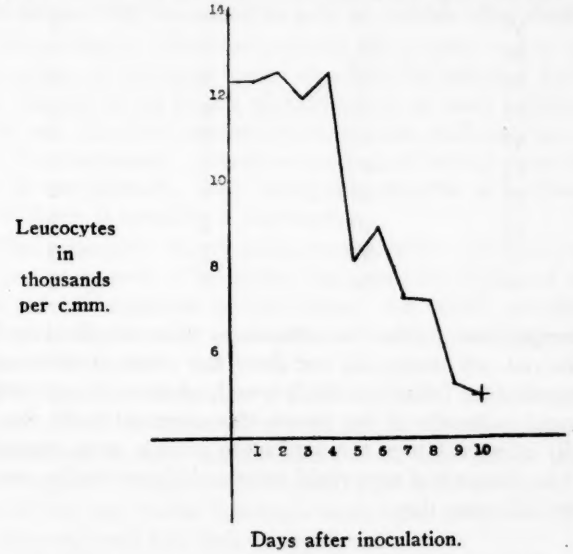

Fig. 5. Showing Total Leucocyte Counts in a case of Feline Enteritis.

whereas Syverton *et al.* (1943) considered it an agranulocytosis with a neutropænia and a relative lymphocytosis.

As a routine examination, the total red and white cell counts were determined, together with a differential leucocyte count. In all cases of feline enteritis which were seen prior to the death of the animal there was a reduction, usually marked, in the total white cell count. In all, blood samples from 15 natural cases and two experimentally infected cases were examined. The fairly spectacular drop in the numbers of leucocytes in an artificially infected case may be seen in Fig. 5.

In one natural case of the disease the white cell count was as low as 900 cells per c.mm. Unfortunately, this was the only count obtained as the kitten died shortly after the blood had been taken. A count of fewer than 6,000 leucocytes, together with any of the symptoms of feline enteritis was regarded as diagnostic. Counts below 7,000 per c.mm. were regarded as justifying a

grave prognosis. It will be recalled that there was no such reduction in numbers of the leucocytes in feline influenza.

The differential leucocyte count also differed from that seen in the respiratory disease. In some cases of feline enteritis the leucocytes in stained smears were so scanty as to make accurate counting impossible. Generally there was a reduction in the numbers of the neutrophils and a consequent lymphocytosis. The differential leucocyte counts for the kitten whose total leucocyte counts are shown in Fig. 5 are as follows:

Days after inoculation	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %
1	56	38	3	3
2	60	25	8	7
3	54	36	5	5
4	57	35	4	4
5	50	37	7	6
6	43	43	7	7
7	35	56	7	2
8	37	58	3	2
9	36	58	3	3
10	36	54	7	3

These blood changes bear a close resemblance to those described in feline agranulocytosis. The red cell counts did not show any constant abnormality.

The *incubation period* for feline enteritis is usually given as being from two to six days. In natural outbreaks of the disease this appeared to be the case. In two experimentally infected kittens the incubation periods were respectively five and two days. The disease is of very rapid onset and kittens healthy one day may be prostrated the following day.

Mortality Rate

Unlike the respiratory disease, feline enteritis has a high mortality rate, especially among young cats. Almost without exception affected cats died, usually within 5-7 days from the commencement of symptoms.

Causal Agent

It has been well established that feline enteritis is of virus origin. Verge and Cristoforoni (1928) first demonstrated the viral nature of the disease and their observations have been repeated by many workers. The condition termed feline agranulocytosis has also been shown to be of virus origin (Hammon and Enders, 1939). In this investigation into feline enteritis, a suspension of spleen from an infected cat was used as a source of virus. The suspension was filtered through a collodion membrane of A.P.D. 0.75μ and the resultant filtrate checked for sterility by the usual means. The disease was reproduced in two kittens but all attempts to infect rabbits, guinea-pigs and mice with the virus failed.

During the course of *post-mortem* examinations on cases of feline enteritis,

bacteriological examinations were made from the usual sites and a variety of pathogenic organisms was isolated. As is usual in virus infections, these secondary bacterial invaders complicate the picture, and no doubt give rise to the variety of symptoms and increase the severity of the lesions. The most constant organism isolated from these cases of feline enteritis was *Bact. coli.* The bacteriological findings in general were very similar to those found in cases of feline influenza.

Pathology

Affected animals which died or which were killed during the acute stages of the disease were examined as soon as possible after death.

Gross lesions.—The most constant lesion noted was an inflammation of the latter portion of the small bowel, especially the terminal part of the duodenum. These changes varied from a slight blush to an acute inflammation. There was usually an abundant muco-purulent exudate and the bowels were invariably empty and contracted. Rarely were changes beyond those of a catarrhal nature noted in the stomach. The term gastro-enteritis as it is sometimes applied to this condition is certainly a misnomer.

The mesenteric lymph nodes were swollen and moist, and in a few cases appeared congested. The spleen was often enlarged and in these cases there was a patchy congestion of this organ. An undue prominence of the lymph follicles was also noted in some cases. The liver and kidneys showed no constant changes of note.

The thoracic viscera were, macroscopically, normal with the exception of two cases which showed congestion of the apical and cardiac lobes.

The bone marrow was examined in all cases and it was invariably found to be changed. In place of the usual rather solid column of red marrow in the shafts of the long bones (kittens) a semi-liquid mass was present. The tissue was very congested and dark in appearance.

It will be noted that these gross lesions resemble those described in feline agranulocytosis.

Microscopic Changes

Portions of affected tissue were fixed in Zenker's fluid and paraffin sections were prepared. The same staining techniques as were used for feline influenza were used in these cases.

Small intestine.—The microscopic changes noted in the small bowel varied a good deal. In all cases there was congestion of the vessels and in a few cases there was evidence of diapedesis and free erythrocytes were present in the exudate in the bowel lumen. The tips of the villi showed desquamation, and in some cases (relatively few) there was extensive necrosis of the bowel mucosa. The number of mucous cells appeared to be increased and there was some cellular infiltration. The cells present were mainly of the mononuclear type; relatively few neutrophils were present.

Careful search was made for possible inclusion bodies and some structures were encountered in some of the cases examined. These bodies were intranuclear in position and were eosinophilic. They varied in size and some nuclei contained up to three of these structures. They were not constantly present and it is interesting to note that Hammon and Enders stated that in cases of feline agranulocytosis, which were examined a few hours after death, inclusion bodies were not always found.

Spleen.—The spleen showed œdema and slight congestion of the vessels. There was an increase in the number of monocytes at the periphery of the germ centres and many of these contained intranuclear eosinophilic bodies. Some of these intranuclear structures may be seen in Fig. 6.

Ileocæcal lymph node.—This node was invariably œdematous with separation of the lymph sinuses. The follicles were usually prominent, but they showed no degeneration resembling that described in feline agranulocytosis (Hammon and Enders, 1943).

Bone marrow.—The marrow was aplastic in type. There were present very few cells, the bulk of the tissue consisted of fat and small hæmorrhages. The cells present were mainly erythrocytes and normoblasts with isolated plasma cells and megakaryocytes. Mature granular leucocytes were absent: a few myeloblasts were present. Such formative tissue as remained was present in small islets.

Summary

A very brief account of the clinical and post-mortem findings in cases of feline enteritis is presented. Special attention is drawn to the blood changes which occur in this condition. There is a definite neutropænia and a relative lymphocytosis. There may be a spectacular drop in the number of circulating white cells.

Acknowledgments

I wish to acknowledge the assistance given by several local practitioners who provided much of the material used here. Also, I wish to thank Dr. G. O. Davies for permission to publish these papers and for helpful advice and criticism throughout, and to my colleagues here and in the Medical School for assistance with the bacteriological work.

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INCLUSION AND CRYSTALLINE BODIES
In Distemper, Fox Encephalitis, Contagious Canine Hepatitis and in Normal Dogs

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A RECENT paper (1949) reviewed some problems of distemper and canine encephalitis and a passing comment was made on the need for a critical re-investigation of the pathology of distemper and other perhaps allied infections. This is a necessity if further advances are to come from work along the lines instigated by MacIntyre *et al.* (1948). It would be advantageous if such pathological work included observations on the nature and distribution of inclusion bodies in experimental, and natural, cases of distemper in dogs and also other infections. The question of rapid diagnosis of distemper is all-important to the clinician but also to the experimentalist; without recourse to ferret inoculation, the identification of inclusion bodies in certain tissues if they were constantly present and absolutely specific might be a great help. It is clear, however, from available publications that there is much discrepancy in the reported findings. Much of this may be due to the fact that there are apparently a number of virus infections in dogs which may simulate canine distemper at least on superficial clinical grounds. Some progress, however, might be achieved if a sufficiently large enough number of cases, and if a wide variety of tissues, were examined critically by histological methods. In such studies what is greatly required is also a definite correlation with clinical and pathological data, for in the past the various aspects along with experimental work with dogs seem to have been regarded as distinct and separate objects of study. The literature reveals that this has undoubtedly been so. This article is based on personal observations* made years ago and with an analysis of more recent observations may be of value to those who have an interest in canine pathology. It may likewise focus attention on some pathological aspects of canine disease which, in England, have not been given the attention deserved.

1. Distemper

In the classical work of Dunkin and Laidlaw (1926) there is no mention of inclusion bodies either in ferret or canine tissues; they were, however, quite aware of their occurrence as is evident from correspondence I had with the late Sir Patrick Laidlaw. He mentioned that no separate account had ever been written by them because they had been originally described by Sanfelice (1915), but Laidlaw regarded them as specific for distemper. Sanfelice, and even others before him, described these inclusions as single, and multiple acidophil bodies (Fig. 1a, b) present in the cytoplasm of a wide variety of cells, e.g., in bronchial and alveolar epithelium, in cells of the reticolo-endothelial system in lymph nodes and spleen, in epithelium of conjunctiva, nasal mucosa, pancreas, skin nea pustules, oviducts and the adrenals, but never in liver or kidney, while intra-nuclear inclusions were seen in nerve cells. Laidlaw tended to believe that the

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inclusion body was a mass of virus particles, like the ectromelia inclusion, although he said he had no evidence to support this view. Since then many workers have described the same bodies in the same, and other, situations but a different degree of significance regarding their pathognomonic importance have been attached to them. Most of the cross references can be obtained in the papers of Green and Evans (1939, a and b) and Watson and Plummer (1942), from which it would appear that no other virus produces both intranuclear and cytoplasmic inclusions. Green and Evans (1939 b) described technique for a rapid identification of these inclusions in scrapings from the bladder, while Watson and Plummer on the basis of experimental work with ferrets, minks and foxes came to the conclusion that they were an aid to microscopic diagnosis but were not pathognomonic. The previous article (Innes, 1949) indicated the doubt surrounding the identity of the viruses said to be responsible for encephalitis in dogs; some scepticism must thus be attached to the diagnostic significance of inclusion bodies in either neurones, or neuroglia, in distemper *without encephalitis* or in demyelinating or inflammatory encephalitis whatever the cause may be (and whether natural or experimental). Sanfelice (1915), Marinesco *et al.* (1933), Green and Evans (1939) and Hurst *et al.* (1943) all illustrate inclusion bodies, some cytoplasmic, some intranuclear, in nerve cells, or glia, while Frauchiger and Walthard (1935), Peters and Yamagiwa (1930) failed to observe them in *encephalitis cases*; MacIntyre *et al.* did not observe them in cases of proven distemper with neuronal damage but without encephalitis. The intranuclear inclusions described by both Hurst and de Monbreun (1937) in ependymal cells (and those by de Monbreun in hepatic cells) are identical with those illustrated by Green for fox encephalitis infection of dogs and by Rubarth (1947) for *Hepatitis contagiosa canis*. Kriesel (1938) describes both intranuclear and cytoplasmic inclusions in lymph nodes, spleen, lungs, etc., and intranuclear bodies in hepatic cells; in experimental fox encephalitis in dogs the intranuclear hepatic inclusions occurred in 100 per cent of puppies but they also occurred in 18 per cent of *distemper puppies*; while cytoplasmic inclusions were found only in 68 per cent of the latter. It would thus be difficult to be certain what are the true facts of their distribution and sites related to distemper in all its various clinical or pathological phases. As stated in the previous article, I often failed to find cytoplasmic inclusions (e.g., bronchial epithelium, nasal mucosa or bladder) when most expected and *vice versa*. I was, however, convinced in the studies made years ago that, in spite of all which has been said about the need for special fixation and staining, when cytoplasmic or intranuclear inclusions are present they can be readily seen after formalin fixation and hæmotoxylin-eosin staining. With Zenker-fixed material and special staining methods they may, however, be much clearer. There is thus room for much investigation along these lines.

2. Fox Encephalitis (Green) and Hepatitis Contagiosa Canis (Rubarth)

The work of Green *et al.* in identifying this fox virus infection has been discussed in the paper in the *Veterinary Record*. Green stated that it was possible to diagnose the infection in dogs by the presence of inclusion bodies in the ependymal cells (if virus was injected cisternally) but more particularly by the presence of intranuclear inclusions in hepatic cells. The same type of latter

inclusion body was seen personally in dogs experimentally infected with the fox virus supplied by Green and is illustrated in Fig. 2. These inclusions were originally described by Cowdry and Scott (1930) in two out of 68 experimental dogs which showed liver necrosis and they discussed a possible relationship to some virus infection because it was obvious there was little connection between their experimental technique and the pathological result. These inclusions were later described by de Monbreun (1937) in his so-called cases of experimental canine distemper; Green and Kriesel thought that this was due to the use of fox encephalitis virus (acquired from an acute natural canine case which was not distemper) and used by de Monbreun for his transmission work on experimental canine distemper encephalitis. Rubarth (1947) clearly identifies a clinical and pathological syndrome in Sweden named by him *Hepatitis contagiosa canis*, and showed the universal presence of the same intranuclear inclusion bodies; he concluded that Green and de Monbreun were working with this virus which had some peculiar pathogenic proclivity for causing liver damage. These claims are now supported by the identification of the same infectious hepatitis in dogs by Coffin (1948), Riser (1948), Bloom (1948) and Jones (1948) in the U.S.A. (and by myself in England, see later paper). It would thus seem that an infection in dogs has existed in some parts of Europe, and the States, for years which has masqueraded no doubt under other clinical names and which simply had not been identified through lack of careful pathological study.

When the intranuclear hepatic cell inclusions were seen personally in 1939 in experimental fox-virus infection in dogs a search was made for similar bodies in livers of spontaneous canine encephalitis cases, with negative results. It was partly on this basis that we concluded that the F.E. virus had no part in the pathogenesis of canine encephalitis as we had seen it in England.

3. Crystalline Inclusions in Hepatic Cells in Normal Dogs

At the same time as the above work was proceeding a search was made of a large number of sections of livers of dogs (normal and diseased but not, peculiarly enough, including any with liver necrosis) as a control measure: no intranuclear hepatic cell inclusions were found. Hence I had little doubt at that time of their specificity, whatever the infection might have been whether that of de Monbreun or Green (and now Rubarth). In one dog (encephalitis case), however, characteristic crystals in the nuclei of hepatic cells were found and in very large number of cells. The crystals were of varying shape—some enlongated, some square, but were, on occasion, round—highly acidophile and might have been mistaken for the intranuclear inclusions shown in Fig. 3 as long as the search had not been prolonged to find crystalline forms. They were then found in normal dogs' livers and after some correspondence with the late Sir Patrick Laidlaw it was discovered that they were structures quite commonly observed in normal dogs. They were originally described by Browicz (1899) after the intravenous injection of hæmoglobin into dogs and since then by many other authors and have been interpreted in different ways. Some workers thought they might be hæmoglobin crystals. All the references dealing with these crystalline bodies are given in a valuable article by Bloom (1943) who made a study of their presence in dogs suffering from a variety of diseases. He

emphasises an original observation by Weatherford (1938), that the Dalmatian dog is exceptional in having fewer crystals and that this animal has a purine metabolism resembling man; this association with a higher uric acid level may be of some significance. He refers to the fact that in man, the blood uric acid level is often elevated in leukæmia and that although in canine leukæmia no clinical pathological studies have been made, a marked reduction in the number of crystals was found.

Conclusions

There is much variation in the recorded descriptions of type, nature and distribution of inclusion bodies in experimental, and natural cases, distemper of dogs and other susceptible animals. A thorough reinvestigation is urgently required, which would relate their occurrence to clinical and pathological stages and phases of the disease. This would be of much value from the point of view of rapid diagnosis. Apart from the observations of Kriesel, it would seem that intranuclear hepatic cell inclusions do not occur in clearly identified natural, or experimental, distemper of dogs or ferrets. They do appear specific for an infection which has been repeatedly seen in dogs in the U.S.A. where it was originally regarded as the fox encephalitis virus infection occurring in dogs, but seems now to be more closely linked with Rubarth's *Hepatitis contagiosa canis* in Sweden. There is thus readily available a diagnostic histological lesion which would serve to identify whether any similar disease to Rubarth's syndrome occurs in dogs in Great Britain. (More recently identified in cases from my pathological collection.) Past history of experimental pathological work with dogs indicates that it would be most important for this to be clarified. In the examination of canine tissues for inclusion bodies the observations of many workers on the frequency of crystalline structures in normal dogs' tissues (and not only in the liver) which are not the result of a specific virus should clearly be borne in mind.

Thanks are due to Major T. C. Jones, Registry of Vet. Path., Army Institute of Pathology, Washington, D.C., for his kindness in presenting me with Fig. 1.

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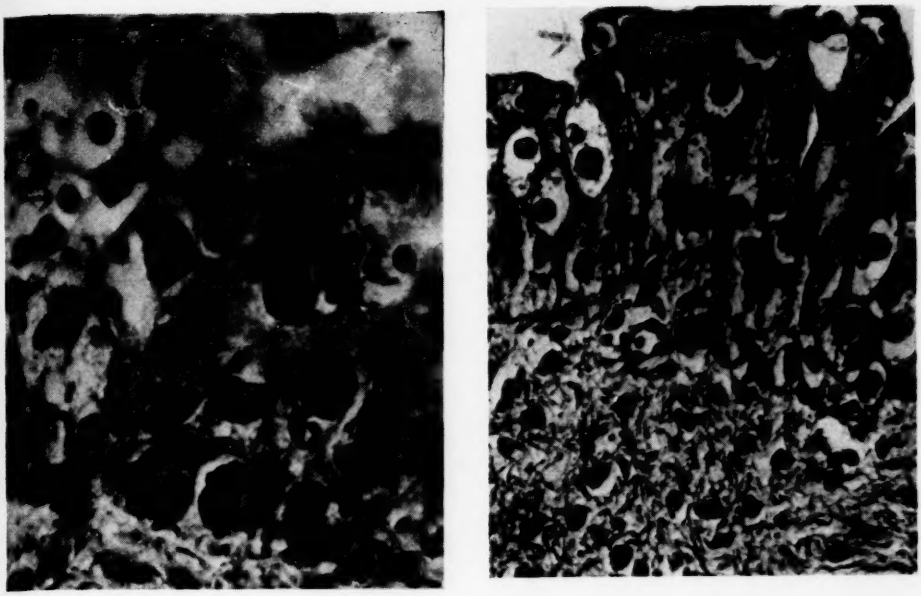

(a) (b)

FIG. 1

Canine distemper.—(a) Bladder; epithelium; natural case; acidophil cytoplasmic inclusions. H.E. X 805 (AC205205). (b) Natural case distemper; Scharr's method X 475 (AC187695).

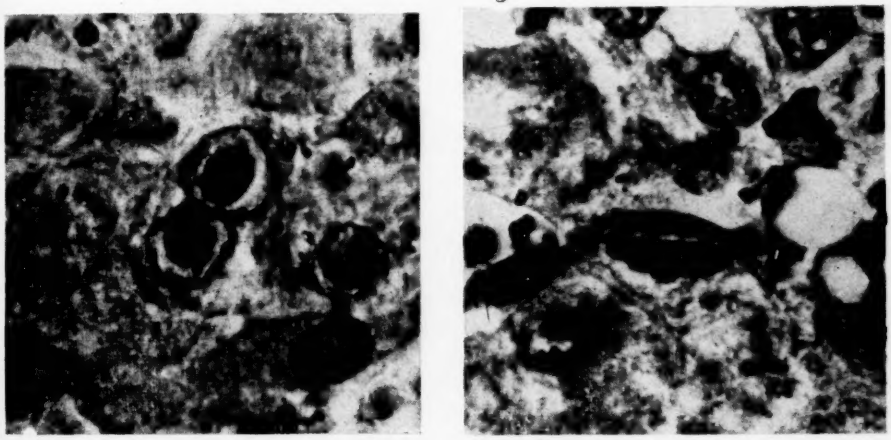

FIG. 2 FIG. 3

Fig. 2.—*Experimental Fox encephalitis, infection in dog:* Injected 1 c.c. Green virus 25.2.38 cisternally; febrile reaction within two days, temp. 105° with tendency to show another rise two days later, like diphasic curve of distemper; on third day stuporous, ataxic gait, off food, fits of screaming; on fourth day, comatose with clonic movements of limbs; death on sixth day. Meningeal infiltrations with some small perivascular demyelinated areas in cerebellum; inclusion bodies in ependymal but not nerve cells; extremely numerous intranuclear inclusions in hepatic cells clearly seen in hæmotoxylin-eosin preparations with margination of chromatin and halo appearance around the inclusion. Hæmotoxylin and eosin. Formal fixation. X about 1000.

Fig. 3.—*Crystalline body in nuclei of hepatic cells of dogs:* The same crystals were seen in normal dogs; this was from a case of disseminated demyelinating encephalomyelitis; note the change of shape in nucleus which coincides with shape of crystal. Hæmotoxylin and eosin, Formalin fixation. X about 1000.

(*Article by J. R. M. Innes on page* 99.)

POLY-VITAMIN THERAPY AND ANTHROPOIDS

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For a number of years vitamin supplements have been given to the animals at London Zoological Gardens. Properly controlled administration is not possible, but it is evident that animals in unnatural surroundings would require these supplements to replace vitamins normally present in their food or made by themselves during periods of prolonged sunshine. The authorities of the Zoo are convinced that the health of the animals has improved since the introduction of vitamin supplements. This applies especially to vitamin D and such conditions as rickets are less frequent than heretofore.

The administration of vitamin D is often difficult as so many of the preparations available are unpalatable, while many of the animals are very "choosey" in their tastes. This applies to the anthropoid apes, while it is these animals which seem to have benefited most by the administration of extra vitamins.

"ABECEDIN" has been found to be taken easily by the anthropoid apes and so is now used as a routine supplement to their diet. On general principles, it would be expected that the vitamins other than D contained in it would be of value to the animals, and, indeed, they seem to be "doing well" on it, but not enough have received the preparation as yet to enable an opinion to be given on this point.

REVIEW

Manual of Veterinary Bacteriology, by Raymond A. Kelser, D.V.M., A.M., Ph.D., and Harry W. Shoening, V.M.D. Fifth Edition. Pp. xi + 767, with 11 Tables and 101 Figures. Baillière, Tindall and Cox, London. Price: 36s. net.

Foods of animal origin—meats, meat food and dairy products—constitute a vital part of the overall food supply and it would have been catastrophic had we not maintained the health of our livestock and jeopardised the production and supply of the huge quantities of food needed during the war years. Because of this there has been an increasing interest in veterinary bacteriology which plays an important rôle in the prevention of animal disease. The publication of textbooks and reference books on this subject are therefore welcome, especially from those who have given considerable help during the critical years.

This book deals with the morphology, physiology and classification of those bacteria of significance in veterinary medicine; descriptions of them constitute the greater part of the work. Those chapters which tell of the methods employed in studying bacteria and the variations which they exhibit deserve special attention. Brief accounts of the pathogenic fungi, protozoa and viruses which are of similar importance are included, as well as descriptions of various serological and hæmatological tests. The book is completed by chapters on the preparation

of biological products and the bacteriological examination of milk and water.

It is essentially an American book with Bergey's nomenclature and emphasis on the micro-organisms which cause animal disease in that country and in those in which the senior author has worked. The bibliography, which is considerable, is indicative not only of the usefulness of the book as a work of reference, but also of the industry of the authors. It is inevitable though in a work of this kind that inaccuracies occur, and one on page 359 must be mentioned: if the toxins are substituted for the antitoxins then we should learn that *Clostridium welchii* type B antitoxin neutralises toxins A, B, C and D; type B toxin is not neutralised by the antitoxins A, B, C and D. One suggests that the determination of the antigenic structure of Brucella as a method of differentiating its members, and how it may be performed, with an adequate description of how one may type the different Salmonella would increase the value of these portions of the book. The extensive description of Erhlich's side chain theory could be shortened and space given to the reticulo-endothelial system; an account of *Babesia bovis* infection in cattle and greater emphasis on the nature of the disease processes caused by micro-organisms described, coupled with fuller sections on diagnosis and control, would also improve it for veterinary surgeons and students. As far as the systematic bacteriology is concerned, it is essentially theoretical in outlook, and yet in those sections dealing with technique there is evidence of the authors' personal experience at the bench. For those engaged in this type of work the book will make its appeal and can be recommended as one which will be constantly consulted, bearing in mind that some of the methods employed here are different from those on the other side of the Atlantic.

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